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                 DKILIT has been renamed APOLLIT
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         Nov 25
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                 TEMA now available on STN
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         May 05
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              MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
              AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
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DT Article English LΑ Objective-To determine apparent seroprevalence of antibodies AΒ against Sarcocystis neurona in a population of domestic cats previously tested for antibodies against Toxoplasma gondii. Design-Cross-sectional study. Sample Population-Serum from 196 domestic cats. Procedure-Banked serum samples submitted to the Michigan State University Animal Health Diagnostic Laboratory for T gondii diagnostic testing were tested for antibodies against S neurona by use of an indirect fluorescent antibody (IFA) test and a western blot test. Submission records were analyzed to determine descriptive statistics and test for associations between positive results of a test for S neurona and other variables in the data set. Results-10 of 196 (5%) samples yielded positive results for antibodies against S neurona by use of western blot analysis, whereas 27 samples yielded positive results by use of the IFA. No association was found between S neurona western blot test results and T gondii test results, age, sex, or the reason for T gondii testing. The S neurona IFA titer was positively and significantly associated with positive results of western blot analysis. Conclusions and Clinical Relevance-Domestic cats are not likely to play a substantial role as intermediate hosts in the natural life cycle of S neurona. Results indicate that natural infection of domestic cats may occur, and small animal practitioners should be aware of this fact when evaluating cats with neurologic disease. The S neurona IFA test had lower specificity than western blot analysis. L14 ANSWER 5 OF 12 WPIDS (C) 2003 THOMSON DERWENT DUPLICATE 3 2001-218486 [22] WPIDS AN2000-571969 [49] CR DNC C2001-065294 TIVaccinating equids against protozoal Sarcocystis neurona infections using unique antigens. DC B04 C06 D16 IN MANSFIELD, L S; MURPHY, A J; ROSSANO, M G; VRABLE, R A PΑ (UNMS) UNIV MICHIGAN STATE CYC 8.8 WO 2001015708 A1 20010308 (200122)* EN PΤ 54p RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW AU 2000071087 A 20010326 (200137) EP 1207889 A1 20020529 (200243) ENR: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI WO 2001015708 A1 WO 2000-US24221 20000831; AU 2000071087 A AU 2000-71087 20000831; EP 1207889 A1 EP 2000-959829 20000831, WO 2000-US24221 20000831 AU 2000071087 A Based on WO 200115708; EP 1207889 A1 Based on WO 200115708 PRAI US 2000-513086 20000224; US 1999-152193P 19990902 WO 200115708 A UPAB: 20020709 NOVELTY - Vaccinating equids against Sarcocystis neurona infections using polypeptide groups of unique 16 (+4) or 30 (+4) antigens of S. neurona, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (a) a vaccine (I) for providing passive immunity to Sarcocystis neurona infection, comprising antibodies against at least one group of a unique 16 (+4) or 30 (+4) antigen of S. neurona;

(b) a vaccine (II) for active immunization of an equid against a S.

neurona infection, comprising at least one group of a unique 16 (+4) or 30 (+4) antigen of S. neurona;

- (c) a vaccine (III) for protecting an equid from S. neurona infection comprising a DNA that encodes at least 1 group of a 16 (+4) kDa antigen and/or a 30 (+4) kDa antigen of S. neurona;
- (d) a method (IV) for vaccinating an equid against a S. neurona infection, comprising:
- (1) providing a recombinant antigen of S. neurona produced from a recombinant microorganism culture (the microorganism contains a DNA that encodes at least one group of a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona; and
 - (2) vaccinating the equid;
- (e) a method (V) for vaccinating an equid against a S. neurona infection, comprising:
- (1) providing a DNA in a carrier solution, a plasmid which encodes at least 1 group of a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of Sarcocystis neurona; and
 - (2) vaccinating the equid with the DNA in the carrier solution:
- (f) a method (VI) of providing passive immunity to a S. neurona infection in a equid, comprising:
- (1) providing **antibodies** against at least 1 group of a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona (the **antibodies** may be monoclonal or polyclonal); and
 - (2) inoculating the equid;
 - (g) a method (VII) for producing a polypeptide, comprising:
- (1) providing a microorganism in a culture containing a DNA encoding a fusion polypeptide comprising at least 1 group of a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona and a polypeptide that facilitates isolation of the fusion polypeptide;
- (2) culturing the microorganism in a culture to produce the fusion polypeptide; and
 - (3) isolating the fusion polypeptide;
 - (h) a method (VIII) for producing an antibody comprising:
- (1) providing a microorganism in a culture containing DNA encoding a fusion polypeptide comprising at least 1 group of a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona and a polypeptide that facilitates isolation of the fusion polypeptide;
- (2) culturing the microorganisms in a culture to produce the fusion polypeptide;
 - (3) isolating the fusion polypeptide;
 - (4) producing the antibody from the polypeptide;
- (i) a monoclonal **antibody** (IX) that selectively binds to a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen;
- (j) an isolated DNA (X) encoding a monoclonal **antibody** that selectively binds to a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen;
- (k) a bacterial clone (XI) containing a plasmid comprising a DNA encoding a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona;
- (1) a vaccine (XII) for an equid comprising an isolated recombinant protein encoded by a cDNA produced from mRNA of S. neurona encoding a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen;
- (m) a vaccine (XIII) for an equid comprising a recombinant virus vector containing DNA encoding a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona;
- (n) a DNA vaccine (XIV) for an equid comprising a plasmid containing DNA encoding a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona; and
- (o) a method (XV) for protecting an equid against S. neurona which comprises providing a vaccine that when injected into the equid causes the equid to produce antibodies against a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona (the antibodies prevent infection by the Sarcocystis neurona).

ACTIVITY - Antiparasitic. No biological data given.

(stratified by the state's opossum (Didelphis virginiana) population and the number of equids on each operation) was selected. Ninety-eight equine-operation owners agreed to participate, and blood collection occurred from late March through October of 1997. Data regarding the 98 farms' feeding and management practices were collected, as well as descriptive data for each of the 1121 individual horses. Serum samples were tested for antibodies to S. neurona using a Western blot test. The true seroprevalence of antibodies specific to S. neurona was estimated to be 60%. Chi-square analysis showed that seroprevalence was lowest in the colder parts of the state that had the fewest opossums (P < 0.0001). In two multivariable logistic-regression analyses with random effects grouped by herd, age and exposure to pasture were associated with increased odds of seropositivity, and feeding of sweet feed (grains mixed with molasses) was associated with decreased odds of testing positive. No association was found between farm size, animal gender, hay types, horse-housing types or exposure to natural surface water and seropositivity.

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L14 ANSWER 8 OF 12 WPIDS (C) 2003 THOMSON DERWENT
                                                       DUPLICATE 5
                        WPIDS
AN
     2000-571969 [53]
     2001-218486 [22]
CR
                        DNC C2000-170452
DNN N2000-423167
     Detection of Sarcocystis neurona, which causes equine
     protozoal myeloencephalitis, in horse serum and cerebrospinal fluid
     comprises identifying a specific antibody-antigen complex via an
     immunoassay.
     B04 C07 D16 S03
DC
     MANSFIELD, L S; MURPHY, A J; ROSSANO, M G;
IN
     VRABLE, R A
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     (UNMS) UNIV MICHIGAN STATE
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     WO 2000049049 A1 20000824 (200053)* EN
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     AU 2000034982 A 20000904 (200103)
                   B1 20020205 (200211)
     US 6344337
ADT WO 2000049049 A1 WO 2000-US4379 20000218; AU 2000034982 A AU 2000-34982
     20000218; US 6344337 B1 Provisional US 1999-120831P 19990219, Provisional
     US 1999-152193P 19990902, US 2000-506630 20000218
FDT AU 2000034982 A Based on WO 200049049
PRAI US 1999-152193P 19990902; US 1999-120831P 19990219; US 2000-506630
     20000218
     WO 200049049 A UPAB: 20020215
AB
     NOVELTY - Detection of Sarcocystis neurona in horses
     by identifying a specific antibody-antigen complex via an
     immunoassay is new.
          DETAILED DESCRIPTION - Detection of Sarcocystis
     neurona in an equine in an immunoassay is improved by reacting a
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biological sample from the horse suspected of harboring the S. neurona with an antibody (Ab) which is selective in binding to an identifying S. neurona antigen (Ag) to form an Ab-Ag complex. INDEPENDENT CLAIMS are also included for the following:

- (1) a kit for detecting S. neurona in a biological sample from an equine;
- (2) monoclonal antibodies against 16 plus or minus 4 kDa or 30 plus or minus 4 kDa antigens of S. neurona; and
- (3) isolated DNA sequences encoding the 16 plus or minus 4 kDa and 30 plus or minus 4 kDa antigens of S. neurona.

USE - The methods and antibodies are useful for detecting

S. neurona (claimed) which causes equine protozoal myeloencephalitis, a neurological disorder in horses. Dwq.0/0ANSWER 9 OF 12 WPIDS (C) 2003 THOMSON DERWENT DUPLICATE 6 2000-292877 [25] WPIDS DNN N2000-219631 DNC C2000-088472 Immunoassay for equine protozoal myeloencephalitis in horses uses specific antibodies to proteins derived from Sarcocystis neurona. B04 C06 D16 S03 MANSFIELD, L S; MURPHY, A J; ROSSANO, M G (UNMS) UNIV MICHIGAN STATE CYC 83 WO 2000017640 A1 20000330 (200025)* EN 26p RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW AU 9954707 A 20000410 (200035) US 6153394 A 20001128 (200063) US 6489148 B1 20021203 (200301) ADT WO 2000017640 A1 WO 1999-US17961 19990809; AU 9954707 A AU 1999-54707 19990809; US 6153394 A US 1998-156954 19980918; US 6489148 B1 Div ex US 1998-156954 19980918, US 2000-569434 20000512 FDT AU 9954707 A Based on WO 200017640; US 6489148 B1 Div ex US 6153394 PRAI US 1998-156954 19980918; US 2000-569434 20000512 WO 200017640 A UPAB: 20000524 NOVELTY - An improved immunoassay for detecting Sarcocystis neurona infection in equines, comprises reacting the Sarcocystis neurona protein with a non-labeled

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antibody to proteins of other Sarcocystis species, before the immunoassay, which inhibits non-specific binding of the labeled antibody, during the immunoassay.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a method for the detection of disease caused by Sarcocystis neurons in equines which comprises:
- (a) isolating fluid from the equine which can contain parasite induced antibodies to Sarcocystis neurona proteins, indicating the presence of the Sarcocystis neurona;
- (b) reacting the fluid with at least one identifying antigen of the Sarcocystis neurons protein bound on a substrate, where the substrate has been blocked with antibodies to Sarcocystis sp. other than Sarcocystis neurons, so that antibodies to Sarcocystis neurona antigen in the fluid are bound to the identifying antigen; and
 - (c) detecting the antibodies bound to the antigen;
- (2) a kit for the detection of disease caused by Sarcocystis neurona comprising in separate containers:
- (a) an identifying antibody able to specifically bind a Sarcocystis neurona protein; and
- (b) a non-labeled antibody which is specific for a second protein of a Sarcocystis sp. other than Sarcocystis neurona; and
- (3) a kit for the detection of disease caused by Sarcocystis neurona in equines comprising:
- (a) a substrate with at least one identifying antigen to the Sarcocystis neurona bound on a surface of the substrate;
 - (b) antibody to a Sarcocystis sp. other than

Sarcocystis neurona; and

(c) at least one reagent for the detection of an antibody in a fluid of the equine which binds to the antigen of Sarcocystis neurona.

USE - The methods and kits are used to detect antibodies to proteins of Sarcocystis neurona, in an equine, (claimed), which causes myeloencephalitis in the equine.

ADVANTAGE - The method uses a non-labeled **antibody** to proteins of other Sarcocystis species to inhibit the non-specific binding of the labeled **antibody**, improving the accuracy of the assay. Dwg.0/2

- L14 ANSWER 10 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
- AN 2001:258224 BIOSIS
- DN PREV200100258224
- TI Immunoassay for equine protozoal myeloencephalitis in horses.
- AU Mansfield, Linda S. (1); Murphy, Alice J.; Rossano, Mary G.
- CS (1) Bath, MI USA
 - ASSIGNEE: Board of Trustees operating Michigan State University
- PI US 6153394 November 28, 2000
- SO Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 28, 2000) Vol. 1240, No. 4, pp. No Pagination. e-file. ISSN: 0098-1133.
- DT Patent
- LA English
- AB An immunoassay for Sarcocystis neurona antibodies in equines is described. The immunoassay uses blocking of Sarcocystis antigens by antibodies to Sarcocystis sp. other than Sarcocystis neurona in connection with the immunoassay.
- L14 ANSWER 11 OF 12 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
- AN 2000-14646 BIOTECHDS
- TI Detection of Sarcocystis neurona, which causes horse protozoan myeloencephalitis, in horse serum and cerebrospinal fluid comprises identifying a specific antibody-antigen complex via an immunoassay;

with use of monoclonal antibodies

- AU Mansfield L S; Rossano M G; Murphy A J; Vrable R A
- PA Univ.Michigan-State
- LO East Lansing, MI, USA.
- PI WO 2000049049 24 Aug 2000
- AI WO 2000-US4379 18 Feb 2000
- PRAI US 990152193 2 Sep 1999; US 1999-120831 19 Feb 1999
- DT Patent
- LA English
- OS WPI: 2000-571969 [53]
- Detection of Sarcocystis neurona, which causes equine protozoan myeloencephalitis in horses by identifying a specific antibody-antigen complex via an immunoassay, is claimed. Also claimed are: a kit for detecting S. neurona in a biological sample from a horse; monoclonal antibodies against antigens of s. neurona; and isolated DNA sequences encoding the antigens of S. neurona. The methods and antibodies are useful for detecting S. neurona which causes horse protozoan myeloencephalitis, a neurological disorder in horses. The labelled antibody against the antigen or the antibody in the antibody-antigen complex is provided for the detecting. The label is chosen from alkaline phosphatase (EC-3.1.3.1), horseradish peroxidase (EC-1.11.1.7), fluorescent compounds, luminescent compounds, colloidal gold and magnetic particles. The label is preferably biotin, which is reacted with peroxidase

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E ROSSANO MARY G/AU
              9 S E3
L3
                E ROSSANO M G/AU
             25 S E3
L4
                E MURPHY ALICE J/AU
             15 S E2 OR E3
L5
                E MURPHY A J/AU
            337 S E3
L6
                E VRABLE RUTH A/AU
             11 S E2 OR E3
L7
                E VRABLE R A/AU
             23 S E2 OR E3
L8
            496 S L1-L8
L9
             39 S L9 AND SARCOCYSTIS NEURONA
L10
L11
             1 S L10 AND (16 KD OR 30 KD)
L12
             51 S L9 AND ANTIBOD?
L13
             26 S L10 AND ANTIBOD?
L14
             12 DUP REM L13 (14 DUPLICATES REMOVED)
=> dup rem 110
PROCESSING COMPLETED FOR L10
             15 DUP REM L10 (24 DUPLICATES REMOVED)
L15
=> d bib ab 1-15
L15 ANSWER 1 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
     2003:159207 BIOSIS
AN
DN
     PREV200300159207
     A herd-level analysis of risk factors for antibodies to
ΤI
     Sarcocystis neurona in Michigan equids.
     Rossano, M. G.; Kaneene, J. B. (1); Marteniuk, J. V.; Banks, B.
ΑU
     D.; Schott, H. C., II; Mansfield, L. S.
     (1) College of Veterinary Medicine, Population Medicine Center, A-109
CS
     Veterinary Medical Center, Michigan State University, East Lansing, MI,
     48824-1314, USA: kaneene@cvm.msu.edu USA
     Preventive Veterinary Medicine, (15 February 2003) Vol. 57, No. 1-2, pp.
SO
     7-13. print.
     ISSN: 0167-5877.
DT
     Article
LΑ
     English
     Equine protozoal myeloencephalitis (EPM) is a neurological disease of
AB
     horses and ponies caused by infection of the central nervous system with
     the protozoan parasite Sarcocystis neurona. A
     herd-level analysis of a cross-sectional study of serum antibodies to S.
     neurona in Michigan equids was conducted, using data collected in 1997 for
     study that included 1121 equids from 98 Michigan horse farms. Our
     objective was to identify specific herd-level risk factors associated with
     seropositivity. We tested associations between herd seroprevalence and
     various farm-management practices (including feed-storage methods and
     wildlife control). Multivariable models were developed for three strata
     based on relative opossum abundance (opossum districts). Herd
     seroprevalence ranged from 0 to 100% (median = 57%); No risk factor was
     significantly associated with herd seroprevalence at P ltoreg 0.05 in all
     opossum districts. Our results suggest that equids living in areas with
     large opossum populations might be infected with S. neurona from multiple
     sources.
L15 ANSWER 2 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN
     2003:67219 BIOSIS
DN
     PREV200300067219
ΤI
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Immunoassay for equine protozoal myeloencephalitis in horses.

Mansfield, Linda S.; Murphy, Alice J. (1);

ΑU

fluorescent antibody (IFA) test and a western blot test. Submission records were analyzed to determine descriptive statistics and test for associations between positive results of a test for S neurona and other variables in the data set. Results-10 of 196 (5%) samples yielded positive results for antibodies against S neurona by use of western blot analysis, whereas 27 samples yielded positive results by use of the IFA. No association was found between S neurona western blot test results and T gondii test results, age, sex, or the reason for T gondii testing. The S neurona IFA titer was positively and significantly associated with positive results of western blot analysis. Conclusions and Clinical Relevance-Domestic cats are not likely to play a substantial role as intermediate hosts in the natural life cycle of S neurona. Results indicate that natural infection of domestic cats may occur, and small animal practitioners should be aware of this fact when evaluating cats with neurologic disease. The S neurona IFA test had lower specificity than western blot analysis.

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L15 ANSWER 5 OF 15 WPIDS (C) 2003 THOMSON DERWENT
                                                       DUPLICATE 3
AN
     2001-218486 [22]
                        WPIDS
CR
     2000-571969 [49]
DNC C2001-065294
TI
     Vaccinating equids against protozoal Sarcocystis neurona
     infections using unique antigens.
DC
     B04 C06 D16
IN
     MANSFIELD, L S; MURPHY, A J; ROSSANO, M G;
     VRABLE, R A
     (UNMS) UNIV MICHIGAN STATE
PA
CYC 88
PΤ
     WO 2001015708 A1 20010308 (200122) * EN
                                              54p
        RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
            NL OA PT SD SE SL SZ TZ UG ZW
         W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB
            GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU
            LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR
            TT UA UG UZ VN YU ZA ZW
     AU 2000071087 A 20010326 (200137)
     EP 1207889
                   A1 20020529 (200243)
                                         ΕN
         R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
            RO SE SI
ADT WO 2001015708 A1 WO 2000-US24221 20000831; AU 2000071087 A AU 2000-71087
     20000831, EP 1207889 A1 EP 2000-959829 20000831, WO 2000-US24221 20000831
FDT AU 2000071087 A Based on WO 200115708; EP 1207889 A1 Based on WO 200115708
PRAI US 2000-513086
                      20000224; US 1999-152193P 19990902
     WO 200115708 A UPAB: 20020709
     NOVELTY - Vaccinating equids against Sarcocystis neurona
```

following:

(a) a vaccine (I) for providing passive immunity to

Sarcocystis neurona infection, comprising antibodies

against at least one group of a unique 16 (+4) or 30 (+4) antigen of S

of S. neurona, is new.

infections using polypeptide groups of unique 16 (+4) or 30 (+4) antiqens

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the

- against at least one group of a unique 16 (+4) or 30 (+4) antigen of S. neurona;
- (b) a vaccine (II) for active immunization of an equid against a S. neurona infection, comprising at least one group of a unique 16 (+4) or 30 (+4) antigen of S. neurona;
- (c) a vaccine (III) for protecting an equid from S. neurona infection comprising a DNA that encodes at least 1 group of a 16 (+4) kDa antigen and/or a 30 (+4) kDa antigen of S. neurona;
- (d) a method (IV) for vaccinating an equid against a S. neurona infection, comprising:
- (1) providing a recombinant antigen of S. neurona produced from a recombinant microorganism culture (the microorganism contains a DNA that

encodes at least one group of a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona; and

- (2) vaccinating the equid;
- (e) a method (V) for vaccinating an equid against a S. neurona infection, comprising:
- (1) providing a DNA in a carrier solution, a plasmid which encodes at least 1 group of a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of Sarcocystis neurona; and
 - $(\bar{2})$ vaccinating the equid with the DNA in the carrier solution;
- (f) a method (VI) of providing passive immunity to a S. neurona infection in a equid, comprising:
- (1) providing antibodies against at least 1 group of a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona (the antibodies may be monoclonal or polyclonal); and
 - (2) inoculating the equid;
 - (g) a method (VII) for producing a polypeptide, comprising:
- (1) providing a microorganism in a culture containing a DNA encoding a fusion polypeptide comprising at least 1 group of a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona and a polypeptide that facilitates isolation of the fusion polypeptide;
- (2) culturing the microorganism in a culture to produce the fusion polypeptide; and
 - (3) isolating the fusion polypeptide;
 - (h) a method (VIII) for producing an antibody comprising:
- (1) providing a microorganism in a culture containing DNA encoding a fusion polypeptide comprising at least 1 group of a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona and a polypeptide that facilitates isolation of the fusion polypeptide;
- (2) culturing the microorganisms in a culture to produce the fusion polypeptide;
 - (3) isolating the fusion polypeptide;
 - (4) producing the antibody from the polypeptide;
- (i) a monoclonal antibody (IX) that selectively binds to a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen;
- (j) an isolated DNA (X) encoding a monoclonal antibody that selectively binds to a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen;
- (k) a bacterial clone (XI) containing a plasmid comprising a DNA encoding a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona;
- (1) a vaccine (XII) for an equid comprising an isolated recombinant protein encoded by a cDNA produced from mRNA of S. neurona encoding a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen;
- (m) a vaccine (XIII) for an equid comprising a recombinant virus vector containing DNA encoding a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona;
- (n) a DNA vaccine (XIV) for an equid comprising a plasmid containing DNA encoding a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona; and
- (o) a method (XV) for protecting an equid against S. neurona which comprises providing a vaccine that when injected into the equid causes the equid to produce antibodies against a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona (the antibodies prevent infection by the Sarcocystis neurona).

ACTIVITY - Antiparasitic.

No biological data given.

MECHANISM OF ACTION - Vaccine.

USE - The vaccines and methods are used for protecting equids against infections by the protozoan parasite Sarcocystis neurona. $\ensuremath{\text{Dwg.0/0}}$

- L15 ANSWER 6 OF 15 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI AN 2001-07735 BIOTECHDS
- TI Vaccinating equids against protozoal Sarcocystis neurona infections using antigens;

Sarcocystis neurona nucleic acid vaccine and recombinant vaccine

AU Mansfield L S; Rossano M G; Murphy A J; Vrable R A

PA Univ.Michigan-State

LO East Lansing, MI, USA.

PI WO 2001015708 8 Mar 2001

AI WO 2000-US24221 31 Aug 2000

PRAI US 2000-513086 24 Feb 2000; US 1999-152193 2 Sep 1999

DT Patent

LA English

OS WPI: 2001-218486 [22]

A method for vaccinating equids against Sarcocystis AΒ neurona infection is claimed. It involves using protein groups of unique 16(+4) or 30(+4) antiqens of S. neurona. Also claimed are: a vaccine (I) for providing passive immunity to Sarcocystis neurona infection; a vaccine (II) for active immunization of an equid against a S. neurona infection; a vaccine (III) for protecting an equid from S. neuronna infection; a method (IV or V) for vaccinating an equid against a S. neurona infection; a method (VI) of providing passive immunity to a S. neurona infection; a method (VII) for producing a protein (e.g. glutathione-transferase); a method (VIII) for producing an antibody; providing a microorganism in a culture containing DNA encoding a fusion protein; a monoclonal antibody (IX); an isolated DNA (X); a bacterial clone (XI); a vaccine (XII) for an equid containing an isolated recombinant protein; a vaccine (XIII or XIV) for an equid containing recombinant virus vector containing DNA; and a method (XV) for protecting an equid against S. neurona. The vaccines and methods are used for protecting equids against infection by the protozoon parasite Srcocystis neurona. (54pp)

L15 ANSWER 7 OF 15 CABA COPYRIGHT 2003 CABI

AN 2001:140181 CABA

DN 20013139279

TI The effects of pyrantel tartrate on Sarcocystis neurona merozoite viability

AU Kruttlin, E. A.; Rossano, M. G.; Murphy, A. J.; Vrable, R. A.; Kaneene, J. B.; Schott, H. C., II; Mansfield, L. S.

- CS Department of Large Animal Clinical Sciences, D201 Veterinary Medicine Center, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA.
- SO Veterinary Therapeutics, (2001) Vol. 2, No. 3, pp. 268-276. 21 ref. ISSN: 1528-3593
- DT Journal
- LA English
- AB S. neurona is the etiologic agent of equine protozoal myeloencephalitis, a neurologic disease of horses. The present study was designed to test the hypothesis that pyrantel tartrate can kill S. neurona merozoites growing in equine dermal cell culture. S. neurona merozoites were exposed to a range of concentrations of pyrantel tartrate or sodium tartrate ranging from 0.001 to 0.01 M. Merozoites were then placed onto equine dermal cell cultures and incubated for 2 weeks to check for viability. At 1 and 2 weeks after inoculation, plaque counts were compared between treatments and, between treatments and controls. Merozoites exposed to concentrations of pyrantel tartrate higher than 0.0025~M~(8.91x10-4~g/ml) did not produce plaques in equine dermal cells, whereas those exposed to similar concentrations of the tartrate salt or medium alone produced significant numbers of plaques. These results that pyrantel tartrate has activity against S. neurona merozoites in vitro and suggest that it may have activity against the sporozoite stage of the parasite found in the equine gut.

- L15 ANSWER 8 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
- AN 2001:169884 BIOSIS
- DN PREV200100169884
- TI Comparison of Sarcocystis neurona isolates derived from horse neural tissue.
- AU Mansfield, L. S. (1); Schott, H. C., II; Murphy, A. J.; Rossano, M. G.; Tanhauser, S. M.; Patterson, J. S.; Nelson, K.; Ewart, S. L.; Marteniuk, J. V.; Bowman, D. D.; Kaneene, J. B.
- CS (1) Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, 48824: mansfie4@cvm.msu.edu USA
- SO Veterinary Parasitology, (26 February, 2001) Vol. 95, No. 2-4, pp. 167-178. print. ISSN: 0304-4017.
- DT Article
- LA English
- SL English
- Sarcocystis neurona is a protozoan parasite that can AB cause neurological deficits in infected horses. The route of transmission is by fecal-oral transfer of sporocysts from opossums. However, the species identity and the lifecycle are not completely known. In this study, Sarcocystis merozoites from eight isolates obtained from Michigan horses were compared to S. neurona from a California horse (UCD1), Sarcocystis from a grackle (Cornell), and five Sarcocystis isolates from feral opossums from Michigan. Comparisons were made using several techniques. SDS-PAGE analysis with silver staining showed that Sarcocystis spp. from the eight horses appeared the same, but different from the grackle isolate. One Michigan horse isolate (MIH6) had two bands at 72 and 25 kDa that were more prominent than the UCD1 isolate and other Michigan horse isolates. Western blot analysis showed that merozoites of eight of eight equine-derived isolates, and the UCD1 S. neurona isolate had similar bands when developed with serum or CSF of an infected horse. Major bands were seen at 60, 44, 30, and 16 kDa. In the grackle (Cornell) isolate, bands were seen at 60, 44, 29, and 16 kDa. DNA from merozoites of each of the eight equine-derived isolates and the grackle-derived isolate produced a 334 bp PCR product (Tanhauser et al., 1999). Restriction fragment length. polymorphism (RFLP) analysis of these horse isolates showed banding patterns characteristic for S. neurona. The grackle (Cornell) isolate had an RFLP banding pattern characteristic of other S. falcatula species. Finally, electron microscopy examining multiple merozoites of each of these eight horse isolates showed similar morphology, which differed from the grackle (Cornell) isolate. We conclude that the eight Michigan horse isolates are S. neurona species and the grackle isolate is an S. falcatula species.
- L15 ANSWER 9 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
- AN 2001:135932 BIOSIS
- DN PREV200100135932
- TI The seroprevalence of antibodies to **Sarcocystis neurona** in Michigan equids.
- AU Rossano, M. G.; Kaneene, J. B. (1); Marteniuk, J. V.; Banks, B. D.; Schott, H. C., II; Mansfield, L. S.
- CS (1) Population Medicine Center, College of Veterinary Medicine, A-109 Veterinary Medical Center, Michigan State University, East Lansing, MI, 48824-1314: kaneene@cvm.msu.edu USA
- SO Preventive Veterinary Medicine, (29 January, 2001) Vol. 48, No. 2, pp. 113-128. print. ISSN: 0167-5877.
- DT Article
- LA English
- SL English

A cross-sectional study of serum antibodies to Sarcocystis AB neurona (the etiologic agent of equine protozoal myeloencephalitis, EPM) was performed on Michigan equids. Our objectives were to determine the seroprevalence of antibodies to S. neurona in Michigan equids and to identify specific risk factors for seropositivity. A random, weighted sample of Michigan horse farms (stratified by the state's opossum (Didelphis virginiana) population and the number of equids on each operation) was selected. Ninety-eight equine-operation owners agreed to participate, and blood collection occurred from late March through October of 1997. Data regarding the 98 farms' feeding and management practices were collected, as well as descriptive data for each of the 1121 individual horses. Serum samples were tested for antibodies to S. neurona using a Western blot test. The true seroprevalence of antibodies specific to S. neurona was estimated to be 60%. Chi-square analysis showed that seroprevalence was lowest in the colder parts of the state that had the fewest opossums (P < 0.0001). In two multivariable logistic-regression analyses with random effects grouped by herd, age and exposure to pasture were associated with increased odds of seropositivity, and feeding of sweet feed (grains mixed with molasses) was associated with decreased odds of testing positive. No association was found between farm size, animal gender, hay types, horse-housing types or exposure to natural surface water and seropositivity.

L15 ANSWER 10 OF 15 WPIDS (C) 2003 THOMSON DERWENT DUPLICATE 6

AN 2000-571969 [53] WPIDS

CR 2001-218486 [22]

DNN N2000-423167

DNC C2000-170452

TI Detection of Sarcocystis neurona, which causes equine protozoal myeloencephalitis, in horse serum and cerebrospinal fluid comprises identifying a specific antibody-antigen complex via an immunoassay.

DC B04 C07 D16 S03

IN MANSFIELD, L S; MURPHY, A J; ROSSANO, M G; VRABLE, R A

(UNMS) UNIV MICHIGAN STATE

CYC 87

PA

PI WO 2000049049 A1 20000824 (200053)* EN 64p

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW

AU 2000034982 A 20000904 (200103) US 6344337 B1 20020205 (200211)

ADT WO 2000049049 A1 WO 2000-US4379 20000218; AU 2000034982 A AU 2000-34982 20000218; US 6344337 B1 Provisional US 1999-120831P 19990219, Provisional US 1999-152193P 19990902, US 2000-506630 20000218

FDT AU 2000034982 A Based on WO 200049049

PRAI US 1999-152193P 19990902; US 1999-120831P 19990219; US 2000-506630 20000218

AB WO 200049049 A UPAB: 20020215

NOVELTY - Detection of **Sarcocystis neurona** in horses by identifying a specific antibody-antigen complex via an immunoassay is new.

DETAILED DESCRIPTION - Detection of **Sarcocystis**neurona in an equine in an immunoassay is improved by reacting a
biological sample from the horse suspected of harboring the S. neurona
with an antibody (Ab) which is selective in binding to an identifying S.
neurona antigen (Ag) to form an Ab-Ag complex.

INDEPENDENT CLAIMS are also included for the following:

(1) a kit for detecting S. neurona in a biological sample from an equine;

- (2) monoclonal antibodies against 16 plus or minus 4 kDa or 30 plus or minus 4 kDa antigens of S. neurona; and
- (3) isolated DNA sequences encoding the 16 plus or minus 4 kDa and 30 plus or minus 4 kDa antigens of S. neurona.

 \mbox{USE} - The methods and antibodies are useful for detecting S. neurona (claimed) which causes equine protozoal myeloencephalitis, a neurological disorder in horses.

Dwg.0/0

L15 ANSWER 11 OF 15 WPIDS (C) 2003 THOMSON DERWENT DUPLICATE 7

AN 2000-292877 [25] WPIDS

DNN N2000-219631 DNC C2000-088472

TI Immunoassay for equine protozoal myeloencephalitis in horses uses specific antibodies to proteins derived from Sarcocystis neurona

DC B04 C06 D16 S03

IN MANSFIELD, L S; MURPHY, A J; ROSSANO, M G

PA (UNMS) UNIV MICHIGAN STATE

CYC 83

PI WO 2000017640 A1 20000330 (200025)* EN 26p

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW

W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW

AU 9954707 A 20000410 (200035)

US 6153394 A 20001128 (200063)

US 6489148 B1 20021203 (200301)

ADT WO 2000017640 A1 WO 1999-US17961 19990809; AU 9954707 A AU 1999-54707 19990809; US 6153394 A US 1998-156954 19980918; US 6489148 B1 Div ex US 1998-156954 19980918, US 2000-569434 20000512

FDT AU 9954707 A Based on WO 200017640; US 6489148 B1 Div ex US 6153394

PRAI US 1998-156954 19980918; US 2000-569434 20000512

AB WO 200017640 A UPAB: 20000524

NOVELTY - An improved immunoassay for detecting Sarcocystis neurona infection in equines, comprises reacting the Sarcocystis neurona protein with a non-labeled antibody to proteins of other Sarcocystis species, before the immunoassay, which inhibits non-specific binding of the labeled antibody, during the immunoassay.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a method for the detection of disease caused by Sarcocystis neurons in equines which comprises:
- (a) isolating fluid from the equine which can contain parasite induced antibodies to **Sarcocystis neurona** proteins, indicating the presence of the **Sarcocystis neurona**;
- (b) reacting the fluid with at least one identifying antigen of the Sarcocystis neurons protein bound on a substrate, where the substrate has been blocked with antibodies to Sarcocystis sp. other than Sarcocystis neurons, so that antibodies to Sarcocystis neurona antigen in the fluid are bound to the identifying antigen; and
 - (c) detecting the antibodies bound to the antigen;
- (2) a kit for the detection of disease caused by **Sarcocystis** neurona comprising in separate containers:
- (a) an identifying antibody able to specifically bind a Sarcocystis neurona protein; and
- (b) a non-labeled antibody which is specific for a second protein of a Sarcocystis sp. other than Sarcocystis neurona; and
- (3) a kit for the detection of disease caused by Sarcocystis neurona in equines comprising:
 - (a) a substrate with at least one identifying antigen to the

Sarcocystis neurona bound on a surface of the substrate;

(b) antibody to a Sarcocystis sp. other than Sarcocystis

neurona; and

(c) at least one reagent for the detection of an antibody in a fluid of the equine which binds to the antigen of Sarcocystis

USE - The methods and kits are used to detect antibodies to proteins of Sarcocystis neurona, in an equine, (claimed), which causes myeloencephalitis in the equine.

ADVANTAGE - The method uses a non-labeled antibody to proteins of other Sarcocystis species to inhibit the non-specific binding of the labeled antibody, improving the accuracy of the assay.

Dwg.0/2

- L15 ANSWER 12 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
- AN 2001:258224 BIOSIS
- DN PREV200100258224
- TI Immunoassay for equine protozoal myeloencephalitis in horses.
- AU Mansfield, Linda S. (1); Murphy, Alice J.; Rossano, Mary G.
- CS (1) Bath, MI USA

ASSIGNEE: Board of Trustees operating Michigan State University

PI US 6153394 November 28, 2000

- Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 28, 2000) Vol. 1240, No. 4, pp. No Pagination. e-file. ISSN: 0098-1133.
- DT Patent
- LA English
- AB An immunoassay for Sarcocystis neurona antibodies in equines is described. The immunoassay uses blocking of Sarcocystis antigens by antibodies to Sarcocystis sp. other than Sarcocystis neurona in connection with the immunoassay.
- L15 ANSWER 13 OF 15 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
- AN 2000-14646 BIOTECHDS
- Detection of Sarcocystis neurona, which causes horse protozoan myeloencephalitis, in horse serum and cerebrospinal fluid comprises identifying a specific antibody-antigen complex via an immunoassay;

with use of monoclonal antibodies

- AU Mansfield L S; Rossano M G; Murphy A J; Vrable R A
- PA · Univ.Michigan-State
- LO East Lansing, MI, USA.
- PI WO 2000049049 24 Aug 2000
- AI WO 2000-US4379 18 Feb 2000
- PRAI US 990152193 2 Sep 1999; US 1999-120831 19 Feb 1999
- DT Patent
- LA English
- OS WPI: 2000-571969 [53]
- Detection of Sarcocystis neurona, which causes equine protozoan myeloencephalitis in horses by identifying a specific antibody-antigen complex via an immunoassay, is claimed. Also claimed are: a kit for detecting S. neurona in a biological sample from a horse; monoclonal antibodies against antigens of s. neurona; and isolated DNA sequences encoding the antigens of S. neurona. The methods and antibodies are useful for detecting S. neurona which causes horse protozoan myeloencephalitis, a neurological disorder in horses. The labelled antibody against the antigen or the antibody in the antibody-antigen complex is provided for the detecting. The label is chosen from alkaline phosphatase (EC-3.1.3.1), horseradish peroxidase (EC-1.11.1.7), fluorescent compounds, luminescent compounds, colloidal gold and magnetic particles. The label is preferably biotin, which is

- LA English
- SL English
- Sarcocystis neurona is a protozoan parasite that AB causes a neurological disease in horses called equine protozoal myeloencephalitis. The route of transmission is speculated to be by fecal-oral transfer of sporocysts shed from opossums. Controversy exists regarding both the natural life cycle for this parasite as well as the species identity of opossum Sarcocystis. To provide stage-specific material for species comparison, 27 opossums from southern Michigan were screened for Sarcocystis spp. sporocysts. Seven opossums were positive for Sarcocystis sporocysts by fecal flotation. A simplified, effective technique for isolation, excystation, and culture of opossum Sarcocystis sp. from mucosal scrapings was developed. All 7 Sarcocystis sp. isolates were successfully cultured to grow long term in equine dermal cells to the merozoite stage. Merozoites were observed between 5 and 15 days after inoculation. In conclusion, opossums shed Sarcocystis sp. sporocysts that may be manipulated to excyst and grow in vitro in equine dermal cell lines to the merozoite stage using the simplified technique described.

=> s sarcocystis neurona
L16 905 SARCOCYSTIS NEURONA

=> s 116 and (12 kd or 13 kd or 14 kd or 15 kd or 16 kd or 17 kd or 18 kd or 19 kd or 20 kd)

7 FILES SEARCHED...

L17 2 L16 AND (12 KD OR 13 KD OR 14 KD OR 15 KD OR 16 KD OR 17 KD OR 18 KD OR 19 KD OR 20 KD)

=> d bib ab 1-2

L17 ANSWER 1 OF 2 MEDLINE

AN 2000152631 MEDLINE

DN 20152631 PubMed ID: 10690772

TI Improvement of western blot test specificity for detecting equine serum antibodies to Sarcocystis neurona.

AU Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C 2nd; Fox J C

CS Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.

SO JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (2000 Jan) 12 (1) 28-32. Journal code: 9011490. ISSN: 1040-6387.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200003

ED Entered STN: 20000330 Last Updated on STN: 20000330 Entered Medline: 20000321

AB Equine protozoal myeloencephalitis (EPM) is a neurological disease of horses and ponies caused by the apicomplexan protozoan parasite <code>Sarcocystis</code> neurona. The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to <code>Sarcocystis</code> cruzi to act as a blocking agent to minimize false-positive results in the western blot test for <code>S</code>. neurona. <code>Sarcocystis</code> neurona merozoites harvested from equine dermal cell culture were heat denatured, and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated proteins were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin

and 0.5% Tween-Tris-buffered saline. Serum samples from 6 horses with S. neurona infections (confirmed by culture from neural tissue) and 57 horses without infections (horses from the Eastern Hemisphere, where S. neurona does not exist) were tested by Western blot. Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the equine samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-kD bands (P<0.001, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking proteins not specific to S. neurona and using reactivity to the 30- and 16-kD bands as the criterion for a positive test.

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L17 ANSWER 2 OF 2 WPIDS (C) 2003 THOMSON DERWENT
     1999-571872 [48]
                       WPIDS
AN
                        DNC C1999-166894
DNN
     N1999-421433
     Biologically pure culture of equine Neospora, used as source of vaccines
     and diagnostic reagents.
     B04 C06 C07 D16 S03
DC
     BARR, B C; CONRAD, P A; MARSH, A E
IN
     (REGC) UNIV CALIFORNIA
PA
CYC 23
     WO 9947927
                  A1 19990923 (199948) * EN
                                              47p
PΤ
        RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
        W: AU CA JP
     AU 9931874 A 19991011 (200008)
                  A 20000606 (200033)
     US 6071737
                  A1 20010103 (200102)
                                        EN
     EP 1064550
         R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
     JP 2002509702 W 20020402 (200225)
                                              47p
ADT WO 9947927 A1 WO 1999-US5754 19990316; AU 9931874 A AU 1999-31874
     19990316; US 6071737 A US 1998-42600 19980316; EP 1064550 A1 EP
     1999-913906 19990316, WO 1999-US5754 19990316; JP 2002509702 W WO
     1999-US5754 19990316, JP 2000-537071 19990316
FDT AU 9931874 A Based on WO 9947927; EP 1064550 Al Based on WO 9947927; JP
     2002509702 W Based on WO 9947927
                      19980316
PRAI US 1998-42600
          9947927 A UPAB: 19991122
     WO
     NOVELTY - Biologically pure culture of equine Neospora, is new.
          DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
     following:
          (a) detecting antibodies (Ab) specifically reactive with equine
     Neospora antigens (Ag) by forming an Ab-Ag complex;
          (b) detecting Neospora by forming a complex with an antibody (Ab1)
```

specifically reactive with Neospora antigen;
(c) detecting Neospora-specific nucleic acid (I) by hybridization with a specific oligonucleotide probe; and

(d) pharmaceutical composition containing equine Neospora immunogen and a carrier.

ACTIVITY - Antiprotozoal.

MECHANISM OF ACTION - Induction of a specific immune response.

USE - Immunogens (optionally expressed from gene therapy vectors)
from equine Neospora are used in vaccines for treatment or prevention of
Neospora infection in horses and other animals. Neospora is a causative
agent of equine protozoal myeloencephalitis (EPM). Detection of
Neospora-specific antigens, antibodies or nucleic acid (by usual
immunoassay or hybridization tests) is used to diagnose infection.
Antibodies (Ab) specific for equine Neospora are used for diagnosis; to
select candidate immunogens for vaccine development; to isolate proteins;
to screen DNA libraries and as therapeutic/prophylactic agents.

ADVANTAGE - Reagents specific for equine Neospora allow differentiation between equine protozoal myeloencephalitis caused by Neospora and Sarcocystis neurona. These pathogens require different treatments and treatment of Neospora is only effective if applied before the parasite has formed cysts. The vaccines also prevent shedding of oocysts by animals known to be infected. Dwg.0/2

=> s 116 and (26 kd or 27 kd or 28 kd or 29 kd or 30 kd or 31 kd or 32 kd or 33 kd or 34 kd)

0 L16 AND (26 KD OR 27 KD OR 28 KD OR 29 KD OR 30 KD OR 31 KD OR L18 32 KD OR 33 KD OR 34 KD)

=> d his

(FILE 'HOME' ENTERED AT 14:49:07 ON 15 MAY 2003)

FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS, LIFESCI, CAPLUS' ENTERED AT 14:49:30 ON 15 MAY 2003

```
E MANSFIELD LINDA S/AU
L1
              30 S E2-E3
                 E MANSFIELD L S/AU
L2
             139 S E3
                 E ROSSANO MARY G/AU
L3
               9 S E3
                 E ROSSANO M G/AU
              25 S E3
L4
                 E MURPHY ALICE J/AU
              15 S E2 OR E3
L5
                 E MURPHY A J/AU
L<sub>6</sub>
             337 S E3
                 E VRABLE RUTH A/AU
L7
              11 S E2 OR E3
                 E VRABLE R A/AU
              23 S E2 OR E3
L8
T.9
             496 S L1-L8
              39 S L9 AND SARCOCYSTIS NEURONA
L10
L11
              1 S L10 AND (16 KD OR 30 KD)
L12
              51 S L9 AND ANTIBOD?
L13
              26 S L10 AND ANTIBOD?
L14
              12 DUP REM L13 (14 DUPLICATES REMOVED)
             15 DUP REM L10 (24 DUPLICATES REMOVED)
L15
L16
             905 S SARCOCYSTIS NEURONA
L17
               2 S L16 AND (12 KD OR 13 KD OR 14 KD OR 15 KD OR 16 KD OR 17 KD
               0 S L16 AND (26 KD OR 27 KD OR 28 KD OR 29 KD OR 30 KD OR 31 KD
L18
```

=> s 116 and molecular weight

5 L16 AND MOLECULAR WEIGHT

=> s 119 and antibod?

3 L19 AND ANTIBOD?

=> d bib ab 1-3

1.20 ANSWER 1 OF 3 MEDLINE ΑN 2002085433 MEDLINE

DN PubMed ID: 11812499 21671299

TI Molecular characterisation of a major 29 kDa surface antigen of Sarcocystis neurona.

ΑU Ellison Siobhan P; Omara-Opyene A Levi; Yowell Charles A; Marsh Antoinette E; Dame John B

CS Department of Pathobiology, University of Florida, P.O. Box 110880, Gainesville, FL 32611-0880, USA.

- SO INTERNATIONAL JOURNAL FOR PARASITOLOGY, (2002 Feb) 32 (2) 217-25. Journal code: 0314024. ISSN: 0020-7519.
- CY England: United Kingdom
- DT Journal; Article; (JOURNAL ARTICLE)
- LA English
- FS Priority Journals
- OS GENBANK-AF397896; GENBANK-AF401682
- EM 200205
- ED Entered STN: 20020129

Last Updated on STN: 20020517 Entered Medline: 20020516

A gene encoding a major 29 kDa surface antigen from Sarcocystis AB neurona, the primary causative agent of equine protozoal myeloencephalitis (EPM), was cloned, sequenced, and expressed as a recombinant protein. A cDNA library was prepared in the expression vector lambda ZAP from polyA+mRNA isolated from S. neurona merozoites cultivated in vitro. Random sequencing of 96 clones identified a clone of an abundant transcript having a translated amino acid sequence with 30% identity to the 31-kDa surface antigen of Sarcocystis muris cyst merozoites. Southern blot analysis indicated that the corresponding gene exists in low copy number within the S. neurona genome, but RNA blot analysis and other data indicated that the gene transcript is highly abundant. The sequence of the cDNA clone encoded an open reading frame specifying a polypeptide of 276 amino acids with a predicted size of 28.7 kDa. The deduced amino acid sequence displayed a hypothetical N-terminal signal peptide sequence followed by a polypeptide containing 12 cysteines. The coding region of the cDNA insert was subcloned into the expression vector pET14b, and a fusion protein expressed. The recombinant polypeptide was recognised by mAb 2A7 and mAb 1631, directed against a 29 kDa native protein found on the surface of cultured merozoites. Antibodies in serum and cerebrospinal fluid from a horse with EPM recognised a 29 kDa native protein of S. neurona merozoites and the 29 kDa $\,$ recombinant protein. This S. neurona surface antigen is named SnSAG1.

- L20 ANSWER 2 OF 3 MEDLINE
- AN 2001354018 MEDLINE
- DN 21127318 PubMed ID: 11223200
- TI Immunoconversion against Sarcocystis neurona in normal and dexamethasone-treated horses challenged with S. neurona sporocysts.
- AU Cutler T J; MacKay R J; Ginn P E; Gillis K; Tanhauser S M; LeRay E V; Dame J B; Greiner E C
- CS Department of Pathobiology, PO Box 100880, College of Veterinary Medicine, University of Florida, Gainesville 32610, USA.
- SO VETERINARY PARASITOLOGY, (2001 Feb 26) 95 (2-4) 197-210.

 Journal code: 7602745. ISSN: 0304-4017.
- CY Netherlands
- DT Journal; Article; (JOURNAL ARTICLE)
- LA English
- FS Priority Journals
- EM 200106
- ED Entered STN: 20010625

Last Updated on STN: 20010625 Entered Medline: 20010621

AB Equine protozoal myeloencephalitis is a common neurologic disease of horses in the Americas usually caused by **Sarcocystis**neurona. To date, the disease has not been induced in horses using characterized sporocysts from Didelphis virginiana, the definitive host. S. neurona sporocysts from 15 naturally infected opossums were fed to horses seronegative for antibodies against S. neurona. Eight horses were given 5x10(5) sporocysts daily for 7 days. Horses were examined for abnormal clinical signs, and blood and cerebrospinal fluid were harvested at intervals for 90 days after the first day of challenge

=> s 116 and kd

3 L16 AND KD L22

=> d bib ab 1-3

MEDLINE ANSWER 1 OF 3 L22

MEDLINE 2000152631 AN

PubMed ID: 10690772 20152631 DN

Improvement of western blot test specificity for detecting equine serum TΙ antibodies to Sarcocystis neurona.

Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C ΑU 2nd; Fox J C

Animal Health Diagnostic Laboratory, The Population Medicine Center, CS Michigan State University, East Lansing 48824, USA.

JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (2000 Jan) 12 (1) 28-32. SO Journal code: 9011490. ISSN: 1040-6387.

United States CY

Journal; Article; (JOURNAL ARTICLE) DT

English LΑ

FS Priority Journals

EΜ 200003

Entered STN: 20000330 ED Last Updated on STN: 20000330 Entered Medline: 20000321

Equine protozoal myeloencephalitis (EPM) is a neurological disease of AB horses and ponies caused by the apicomplexan protozoan parasite Sarcocystis neurona. The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to Sarcocystis cruzi to act as a blocking agent to minimize false-positive results in the western blot test for S. neurona. Sarcocystis neurona merozoites harvested from equine dermal cell culture were heat denatured, and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient Separated proteins were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 horses with S. neurona infections (confirmed by culture from neural tissue) and 57 horses without infections (horses from the Eastern Hemisphere, where S. neurona does not exist) were tested by Western blot. Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the equine samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-kDbands (P<0.001, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking proteins not specific to S. neurona and using reactivity to the 30- and 16-kD bands as the criterion for a positive test.

- MEDLINE ANSWER 2 OF 3 L22
- MEDLINE 93222344 AN
- PubMed ID: 8466988 DN
- Equine protozoal myeloencephalitis: antigen analysis of cultured TI Sarcocystis neurona merozoites.
- Granstrom D E; Dubey J P; Davis S W; Fayer R; Fox J C; Poonacha K B; Giles ΑU
- Department of Veterinary Science, University of Kentucky, Lexington CS 40546-0099.

```
JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (1993 Jan) 5 (1) 88-90.
SO
     Journal code: 9011490. ISSN: 1040-6387.
CY
     United States
DT
     Journal; Article; (JOURNAL ARTICLE)
LΑ
     English
FS
     Priority Journals
EM
     199305
ED
     Entered STN: 19930521
     Last Updated on STN: 19930521
     Entered Medline: 19930510
AB
     Antigens of cultured Sarcocystis neurona merozoites
     were examined using immunoblot analysis. Blotted proteins were probed
     with S. cruzi, S. muris, and S. neurona antisera produced in rabbits, S.
     fayeri (pre- and post-infection) and S. neurona (pre- and
     post-inoculation) sera produced in horses, immune sera from 7
     histologically confirmed cases of equine protozoal myeloencephalitis
     (EPM), and pre-suckle serum from a newborn foal. Eight proteins, 70, 24,
     23.5, 22.5, 13, 11, 10.5, and 10 Kd, were detected only by S.
     neurona antiserum and/or immune serum from EPM-affected horses. Equine
     sera were titered by the indirect immunofluorescent antibody (IFA) method
     using air-dried, cultured S. neurona merozoites. Anti-Sarcocystis IFA
     titers were found in horses with or without EPM. Serum titers did not
     correspond to the number of specific bands recognized on immunoblots.
L22 ANSWER 3 OF 3 WPIDS (C) 2003 THOMSON DERWENT
                       WPIDS
AN
     1999-571872 [48]
                        DNC C1999-166894
DNN
    N1999-421433
     Biologically pure culture of equine Neospora, used as source of vaccines
ΤI
     and diagnostic reagents.
DC
     B04 C06 C07 D16 S03
IN
     BARR, B C; CONRAD, P A; MARSH, A E
PA
     (REGC) UNIV CALIFORNIA
CYC 23
                  A1 19990923 (199948)* EN
PΙ
     WO 9947927
                                              47p
        RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
        W: AU CA JP
     AU 9931874 A 19991011 (200008)
     US 6071737 A 20000606 (200033)
                  A1 20010103 (200102)
     EP 1064550
                                        EN
         R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
     JP 2002509702 W 20020402 (200225)
                                             47p
ADT WO 9947927 A1 WO 1999-US5754 19990316; AU 9931874 A AU 1999-31874
     19990316; US 6071737 A US 1998-42600 19980316; EP 1064550 A1 EP
     1999-913906 19990316, WO 1999-US5754 19990316; JP 2002509702 W WO
     1999-US5754 19990316, JP 2000-537071 19990316
FDT AU 9931874 A Based on WO 9947927; EP 1064550 A1 Based on WO 9947927; JP
     2002509702 W Based on WO 9947927
PRAI US 1998-42600
                     19980316
          9947927 A UPAB: 19991122
     NOVELTY - Biologically pure culture of equine Neospora, is new.
          DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
     following:
          (a) detecting antibodies (Ab) specifically reactive with equine
     Neospora antigens (Ag) by forming an Ab-Ag complex;
          (b) detecting Neospora by forming a complex with an antibody (Ab1)
     specifically reactive with Neospora antigen;
          (c) detecting Neospora-specific nucleic acid (I) by hybridization
     with a specific oligonucleotide probe; and
          (d) pharmaceutical composition containing equine Neospora immunogen
     and a carrier.
          ACTIVITY - Antiprotozoal.
          MECHANISM OF ACTION - Induction of a specific immune response.
          USE - Immunogens (optionally expressed from gene therapy vectors)
```

from equine Neospora are used in vaccines for treatment or prevention of Neospora infection in horses and other animals. Neospora is a causative agent of equine protozoal myeloencephalitis (EPM). Detection of Neospora-specific antigens, antibodies or nucleic acid (by usual immunoassay or hybridization tests) is used to diagnose infection. Antibodies (Ab) specific for equine Neospora are used for diagnosis; to select candidate immunogens for vaccine development; to isolate proteins; to screen DNA libraries and as therapeutic/prophylactic agents.

ADVANTAGE - Reagents specific for equine Neospora allow differentiation between equine protozoal myeloencephalitis caused by Neospora and Sarcocystis neurona. These pathogens require different treatments and treatment of Neospora is only effective if applied before the parasite has formed cysts. The vaccines also prevent shedding of oocysts by animals known to be infected. Dwg.0/2

```
=> s 116 and antibod?
L23
          343 L16 AND ANTIBOD?
=> s 123 and (treat? or therap?)
   4 FILES SEARCHED...
           50 L23 AND (TREAT? OR THERAP?)
L24
=> dup rem 124
PROCESSING COMPLETED FOR L24
            23 DUP REM L24 (27 DUPLICATES REMOVED)
L25
=> d bib ab 1-23
    ANSWER 1 OF 23 CAPLUS COPYRIGHT 2003 ACS
     2002:946917 CAPLUS
AN
DN
    138:3686
ΤI
    Monoclonal antibodies to Sarcocystis neurona
     and uses thereof
TN
    Marsh, Antoinette
PΔ
    USA
    U.S. Pat. Appl. Publ., 14 pp., which
SO
    CODEN: USXXCO
DT
    Patent
LΑ
    English
FAN.CNT 1
     PATENT NO.
                   KIND DATE
                                        APPLICATION NO. DATE
     -----
    US 2002187517 A1 20021212
                                         US 2002-140754. 20020507
PRAI US 2001-293603P P
                           20010524
    US 2001-297810P P
                          20010.612
AB
     The present invention is directed to particular monoclonal
     antibodies (2A7-18 and 2G5-2) that find use in the identification
     and purifn. of S. neurona and related antigens. In particular, these
     antibodies permit the diagnosis of Sarcocystis related diseases
     such as equine protozoal myeloencephalitis (EPM).
    ANSWER 2 OF 23 CAPLUS COPYRIGHT 2003 ACS
L25
     2002:638328 CAPLUS
AN
DN
     137:151587
     Use of SAG-1 gene of Sarcocystis neurona for
ΤI
     diagnostic tests and vaccines for equine protozoal myeloencephalitis
IN
     Dame, John B.; Ellison, Siobhan P.; Yowell, Charles A.
PA
SO
    U.S. Pat. Appl. Publ., 21 pp.
     CODEN: USXXCO
DT
    Patent
```

LA English

FAN.CNT 1

PATENT NO. KIND DATE APPLICATION NO. DATE

PI US 2002115828 A1 20020822 US 2001-962993 20010924

PRAI US 2000-234676P P 20000922

- AB A gene encoding a 29 kilodalton protein found on the surface of merozoite stage S. neurona has been cloned and sequenced. The protein encoded by this gene, termed SnSAG-1, is an immunodominant antigen recognized on protein blots. Methods for using nucleic acids and polypeptides relating to SnSAG-1 in diagnostic tests and vaccine development are disclosed. Claimed sequences were not present at the time of publication.
- L25 ANSWER 3 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
- AN 2003:112305 BIOSIS
- DN PREV200300112305
- TI Experimental induction of equine protozoan myeloencephalitis (EPM) in the horse: Effect of Sarcocystis neurona sporocyst inoculation dose on the development of clinical neurologic disease.
- AU Sofaly, C. D. (1); Reed, S. M.; Gordon, J. C. (1); Dubey, J. P.; Oglesbee, M. J.; Njoku, C. J. (1); Grover, D. L. (1); Saville, W. J. A. (1)
- CS (1) Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1092, USA: saville.4@osu.edu USA
- SO Journal of Parasitology, (December 2002, 2002) Vol. 88, No. 6, pp. 1164-1170. print.

 ISSN: 0022-3395.
- DT Article
- LA English
- The effect of inoculation dose of Sarcocystis neurona AB sporocysts on the development of clinical neurologic disease in horses was investigated. Twenty-four seronegative weanling horses were subjected to the natural stress of transport and then randomly assigned to 6 treatment groups of 4 horses each. Horses were then immediately inoculated with either 102, 103, 104, 105, or 106 S. neurona sporocysts or placebo using nasogastric tube and housed indoors. Weekly neurologic examinations were performed by a blinded observer. Blood was collected weekly for antibody determination by Western blot analysis. Cerebrospinal fluid was collected before inoculation and before euthanasia for S. neurona antibody determination. Horses were killed and necropsied between 4 and 5 wk after inoculation. Differences were detected among dose groups based on seroconversion times, severity of clinical neurologic signs, and presence of microscopic lesions. Seroconversion of challenged horses was observed as early as 14 days postinfection in the 106 sporocyst dose group. Mild to moderate clinical signs of neurologic disease were produced in challenged horses from all groups, with the most consistent signs seen in the 106 sporocyst dose group. Histologic lesions suggestive of S. neurona infection were detected in 4 of the 20 horses fed sporocysts. Parasites were not detected in equine tissues by light microscopy, immunohistochemistry, or bioassay in gamma-interferon gene knockout mice. Control horses remained seronegative for the duration of the study and had no histologic evidence of protozoal infection.
- L25 ANSWER 4 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
- AN 2002:348434 BIOSIS
- DN PREV200200348434
- TI Reduced levels of nitric oxide metabolites in cerebrospinal fluid are associated with equine protozoal myeloencephalitis.
- AU Njoku, Chinedu J.; Saville, William J. A.; Reed, Stephen M.; Oglesbee, Michael J.; Rajala-Schultz, Paivi J.; Stich, Roger W. (1)
- CS (1) Department of Veterinary Preventive Medicine, The Ohio State

University, 1900 Coffey Rd., Columbus, OH, 43210-1092: stich.2@osu.edu USA Clinical and Diagnostic Laboratory Immunology, (May, 2002) Vol. 9, No. 3, SO pp. 605-610. print. ISSN: 1071-412X.

DTArticle

English LΑ AB

- Equine protozoal myeloencephalitis (EPM) is a disease of horses that is primarily associated with infection with the apicomplexan Sarcocystis neurona. Infection with this parasite alone is not sufficient to induce the disease, and the mechanism of neuropathogenesis associated with EPM has not been reported. Nitric oxide (NO) functions as a neurotransmitter, a vasodilator, and an immune effector and is produced in response to several parasitic protozoa. The purpose of this work was to determine if the concentration of NO metabolites (NOx-) in the cerebrospinal fluid (CSF) is correlated with the development of EPM. CSF NOx- levels were measured before and after transport-stressed, acclimated, or dexamethasone-treated horses (n = 3 per group) were experimentally infected with S. neurona sporocysts. CSF NOx- levels were also compared between horses that were diagnosed with EPM after natural infection with S. neurona and horses that did not have clinical signs of disease or that showed no evidence of infection with the parasite (n = 105). Among the experimentally infected animals, the mean CSF NOx- levels of the transport-stressed group, which had the most severe clinical signs, was reduced after infection, while these values were found to increase after infection in the remaining groups that had less severe signs of EPM. Under natural conditions, horses with EPM (n = 65) had a lower mean CSF NOx- concentration than clinically normal horses with antibodies (Abs) against S. neurona (n = 15) in CSF, and horses that developed ataxia (n = 81) had a significantly lower mean CSF NOxconcentration than horses that did not have neurologic signs (n = 24). In conclusion, lower CSF NOx- levels were associated with clinical EPM, suggesting that measurement of CSF NOx- levels could improve the accuracy of diagnostic tests that are based upon detection of S. neurona-specific Abs in CSF alone and that reduced NO levels could be causatively related to the development of EPM.
- ANSWER 5 OF 23 CABA COPYRIGHT 2003 CABI L25
- 2002:114296 CABA AN
- DN 20023070361
- Folate deficiency during treatment with orally administered ΤI folic acid, sulphadiazine and pyrimethamine in a horse with suspected equine protozoal myeloencephalitis (EPM)
- Piercy, R. J.; Hinchcliff, K. W.; Reed, S. M. AU
- Department of Clinical Veterinary Science, The Ohio State University, 601 CS Vernon L. Tharp Street, Columbus, OH 43210, USA.
- Equine Veterinary Journal, (2002) Vol. 34, No. 3, pp. 311-316. 35 ref. SO ISSN: 0425-1644
- DTJournal
- LΑ English
- A 6-year-old Quarter Horse show-mare with tail block suffering from AB dysphagia and ataxia believed to be the cause of equine protozoal myeloencephalitis (EPM) is presented at the Department of Clinical Veterinary Science, Ohio State University, Columbus Ohio, USA [date not given]. Sulfadiazine (14.7-44.4 mg/kg), pyrimethamine (0.7-2.2 mg/kg) and folic acid (9.6 mg, q. 12 h) was given to the animal for 9 months. Physical examination revealed ulceration and inflammation of the tongue, and a draining abscess and localized subcutaneous swelling at the dorsal aspect of the tail head. Haematology and serum biochemistry revealed anaemia and leukopenia, neutropenia, lymphopenia, low total CO2, hyperbilirubinaemia, and elevated creatinine and albumin. Previous medications were stopped and lactated Ringer's solution (120 ml/kg/day, i.v.), potassium penicillin (22 000 IU/kg, q 6 h, i.v.), gentamicin (Gentocin, 6.6 mg/kg, i.v.) and folic acid (0.11 mg/kg, i.v.) were

administered to the horse. Ater 6 days of treatment, cerebrospinal fluid and serum were submitted for Sarcocystis neurona antibodies. On day 8, there was complete healing of lingual ulcers and penicillin and gentamicin were discontinued while ceftiofur (Naxcel, 2.2 mg/kg q 12 h, i.m.) was administered. Four days later, the presence of S. neurona antibodies was confirmed, thus, supporting the diagnosis of EPM, and diclazuril (Clinacox, 5 mg/kg q 24 h, p.o.) was administered. After 68 days, the physical, oral and neurological examinations were declared all normal. It is concluded that folic acid supplementation does not prevent the development of folate deficiency and that it is not recommended to supplement folic acid to

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horses being treated with dihydrofolate reductase inhibitors.
L25 ANSWER 6 OF 23 WPIDS (C) 2003 THOMSON DERWENT
     2002-049244 [06]
                        WPIDS
AN
DNC C2002-013806
     Vaccine useful for preventing or ameliorating equine protozoal
ΤI
     myeloencephalitis disease, comprises inactivated Sarcocystis
     neurona cells and/or Neospora hughesi cells, antigens, DNA derived
     from the cells or their mixtures.
     B04 C06 D16
DC
     BIGBIE, R B; NG, T K; WHALEN, J W
IN
     (AMHP) AMERICAN HOME PROD CORP; (AMHP) WYETH
PA
CYC
     WO 2001080885 A2 20011101 (200206) * EN
PΙ
                                              31p
        RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
            NL OA PT SD SE SL SZ TR TZ UG ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ
            LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD
            SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
     AU 2001051761 A 20011107 (200219)
     US 2002041886 A1 20020411 (200227)
     EP 1276499
                  A2 20030122 (200308)
                                         EN
         R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
            RO SE SI TR
     BR 2001010232 A 20030121 (200309)
ADT WO 2001080885 A2 WO 2001-US40527 20010413; AU 2001051761 A AU 2001-51761
     20010413; US 2002041886 Al Provisional US 2000-199435P 20000425,
     Provisional US 2001-278695P 20010326, US 2001-840485 20010423; EP 1276499
     A2 EP 2001-925175 20010413, WO 2001-US40527 20010413; BR 2001010232 A BR
     2001-10232 20010413, WO 2001-US40527 20010413
FDT AU 2001051761 A Based on WO 200180885; EP 1276499 A2 Based on WO
     200180885; BR 2001010232 A Based on WO 200180885
PRAI US 2001-278695P 20010326; US 2000-199435P 20000425; US 2001-840485
     20010423
AB
     WO 200180885 A UPAB: 20020128
     NOVELTY - An immunogenically active component (I) comprising a merozoite
     antibody inducing, inactivated Sarcocystis
     neurona cells, tachyzoite antibody inducing, inactivated
     Neospora hughesi cells, a merozoite or tachyzoite antibody
     inducing antigen derived from the cells, DNA derived from the cells
     capable of inducing a merozoite or tachyzoite antibody immune
     response or their mixture, is new.
          DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
     following:
```

- (1) a vaccine composition comprising (I), a pharmacologically
- acceptable carrier, and an immunogenicaly stimulating adjuvant; (2) cell culture propagation (III) of S.neurona or N.hughesi protozoan parasite, comprising:
 - (i) growing a monolayer of cells having a confluency of 80-100%;
 - (ii) re-feeding the cells with supplemented growth media;
 - (iii) inoculating the cells with merozoites or tachyzoites;

- (iv) holding the inoculated cells for 4-12 days; and
- (v) decanting the supplemented growth media from the inoculated cells and refeeding the cells a second time with supplemented growth media; and
- (3) preventing or ameliorating an EPM disease in equines, comprising administering an immunogenically active component selected from the following:
 - (i) merozoite antibody inducing, inactivated

Sarcocystis neurona cells;

- (ii) tachyzoite antibody inducing, inactivated Neospora
 hughesi cells;
- (iii) a merozite or tachyzoite **antibody** inducing antigen derived from the cells;
- (iv) DNA derived from the cells, capable of inducing a merezoite or tachyzoite antibody immune response; or
 - (v) a mixture of the above.

ACTIVITY - Neuroprotective; Antiinflammatory. No biological data was provided.

MECHANISM OF ACTION - Vaccine. One group of horses were administered 1 multiply 105 merozoites/dose of the vaccine, a second group of 21 horses were administered vaccine blended at 1 multiply 106 merozoites/dose, a third group of 10 horses were administered vaccine at 1 multiply 107 merozoites/dose, and a fourth of group of 10 horses were maintained as non-vaccinated environmental controls. **Treated** horses from all groups showed significant increases in **antibodies** to S.neurona merozoites while the control horses maintained a low to non-existent **antibody** level.

USE - (I) and (II) are useful for prevention or amelioration of equine protozoal myeloencephalitis (EPM) disease in equines. (III) is useful for the propagation of cells such as equine dermal, maiden darby bovine kidney, African green monkey kidney, canine monocyte, mouse monocyte, fetal rhesus monkey kidney, feline kidney, maiden darby canine kidney and baby hamster kidney cells (claimed).

Dwg:0/0

L25 ANSWER 7 OF 23 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

AN 2002-04497 BIOTECHDS

Vaccine useful for preventing or ameliorating equine protozoal myeloencephalitis disease, comprises inactivated Sarcocystis neuroma cells and/or Neospora hughesi cells, antigens, DNA derived from the cells or their mixtures;

horse protozoon myeloencephalitis disease therapy suing a recombinant vaccine or a nucleic acid vaccine

AU Bigbie R B; Ng T K; Whalen Jr J W

PA American-Home-Prod.

LO Madison, NJ, USA.

PI WO 2001080885 1 Nov 2001

AI WO 2001-US40527 13 Apr 2001

PRAI US 2001-278695 26 Mar 2001; US 2000-199435 25 Apr 2000

DT Patent

LA English

OS WPI: 2002-049244 [06]

An immunogenically active component (I) having a merozoite
antibody inducing, inactivated Sarcocystis
neurona cells, tachyzoite antibody inducing,
inactivated Neospora hughesi cells, a merozoite or tachyzoite
antibody inducing antigen derived from the cells, DNA derived
from the cells capable of inducing a merozoite or tachyzoite
antibody immune response or their mixture, is new. Also claimed
are: a vaccine composition comprising (I), a pharmacologically acceptable
carrier, and an immunogenically stimulating adjuvant; cell culture
propagation (III) of S. neurona or N. hughesi protozoan parasite,
involving growing a monolayer of cells, re-feeding with supplemented
growth medium, inoculating with merozoites or tachyzoites, holding for

4-12 days and decanting the supplemented growth medium from the inoculated cells and refeeding a second time with supplemented growth medium; and preventing or ameliorating an EPM disease in horses. (III) is useful for the propagation of cells such as horse dermal, cattle kidney, African green monkey kidney, dog monocyte, mouse monocyte, fetal rhesus monkey kidney, feline kidney, dog kidney and baby hamster kidney cells. (31pp)

- L25 ANSWER 8 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
- AN 2001075088 EMBASE
- TI Migration and development of **Sarcocystis neurona** in tissues of interferon gamma knockout mice fed sporocysts from a naturally infected opossum.
- AU Dubey J.P.
- CS J.P. Dubey, United States Dept. of Agriculture, Parasite Biol., Epidem./Syst. Lab., Animal and Natural Resources Inst, Beltsville, MD 20705-2350, United States. jdubey@anri.barc.usda.gov
- SO Veterinary Parasitology, (26 Feb 2001) 95/2-4 (341-351).

Refs: 19

ISSN: 0304-4017 CODEN: VPARDI

- PUI S 0304-4017(00)00401-5
- CY Netherlands
- DT Journal; Article
- FS 004 Microbiology
 - 005 General Pathology and Pathological Anatomy
- LA English
- SL English
- Migration and development of Sarcocystis neurona was AB studied in 50 gamma interferon knockout mice fed graded doses of S. neurona sporocysts from the intestine of a naturally infected opossum. Mice were examined at necropsy 1-62 days after feeding sporocysts (DAFS). All tissue sections were reacted with anti-S. neurona-specific polyclonal rabbit serum in an immunohistochemical (IHC) test. Between 1 and 3 DAFS, organisms were seen mainly in intestines. Between 4 and 11 DAFS, organisms were seen in several visceral tissues. Beginning with 13 DAFS, schizonts and merozoites were present in sections of brains of all infected mice. All regions of the brain were parasitized but the hind brain was most severely affected. S. neurona was found in the spinal cord of all 10 mice examined 22-30 DAFS. Of the 28 infected mice examined 20-62 DAFS, S. neurona was found in the brains of all 28, lungs of 14, hearts of 8 and eyes of 3. More organisms were seen in IHC-stained sections than in sections stained with hematoxylin and eosin. Treatment of tissues with glutaraldehyde, Karnovsky fixative, and ethylene diamino tetra acetic acid (EDTA, used for decalcification) did not affect staining of organisms by IHC.
- L25 ANSWER 9 OF 23 MEDLINE

DUPLICATE 3

- AN 2001511543 MEDLINE
- DN 21442379 PubMed ID: 11558662
- TI Suspected protozoal myeloencephalitis in a two-month-old colt.
- AU Gray L C; Magdesian K G; Sturges B K; Madigan J E
- CS Veterinary Medical Teaching Hospital, Large Animal Clinic, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis 95616, USA.
- SO VETERINARY RECORD, (2001 Sep 1) 149 (9) 269-73. Journal code: 0031164. ISSN: 0042-4900.
- CY England: United Kingdom
- DT Journal; Article; (JOURNAL ARTICLE)
- LA English
- FS Priority Journals
- EM 200201
- ED Entered STN: 20010918

Last Updated on STN: 20020125

horse would have clinical improvement. The likelihood of survival among horses with EPM was lower among horses with more severe clinical signs and higher among horses that improved after EPM was diagnosed. Conclusions and Clinical Relevance: **Treatment** of horses with EPM is indicated in most situations; however, severity of clinical signs should be taken into consideration when making **treatment** decisions. Response to **treatment** is an important indicator of survival.

- L25 ANSWER 14 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
- AN 2000:339710 BIOSIS
- DN PREV200000339710
- TI Detection of Sarcocystis neurona in the brain of a Grant's zebra (Equus burchelli bohmi.
- AU Marsh, Antoinette E. (1); Denver, Mary; Hill, Frazer I.; McElhaney, M. R.; Trupkiewicz, J. G.; Stewart, James; Tell, Lisa
- CS (1) Department of Veterinary Pathobiology, University of Missouri, Columbia, MO, 65211 USA
- SO Journal of Zoo and Wildlife Medicine, (March, 2000) Vol. 31, No. 1, pp. 82-86. print.
 ISSN: 1042-7260.
- DT Article
- LA English
- SL English
- AB An 8-yr-old intact male Grant's zebra (Equus burchelli bohmi) was referred to the Veterinary Medical Teaching Hospital of the University of California-Davis after being found in the owner's pasture obtunded and in lateral recumbency. The animal was hypothermic, weak, and unwilling to rise. There was no evidence of trauma, and the zebra had seemed normal the preceding evening. There was no extensor rigidity, and cranial nerve reflexes were normal. Flexor and extensor reflexes were weak upon initial examination. A complete blood count and serum biochemistry analysis revealed a mild leukocytosis, hyperfibrinogenemia, hypoglycemia, hyponatremia, hypochloremia, hypocalcemia, and hypoalbuminemia. Urinalysis was normal, and a urine toxicology screen for alkaloids was negative. No toxic substance was found in the hay or pasture grasses although the owner reported the presence of yellow star thistle and mushrooms in the pasture. The cerebrospinal fluid cytologic and biochemical analyses were normal, but antibodies to Sarcocystis neurona were detected. The zebra died despite aggressive supportive therapy

over a 4-day period. The necropsy demonstrated severe gastrointestinal nematodiasis that could account for hypoalbuminemia and electrolyte abnormalities. Histopathologic examination of the nervous system revealed focal areas of perivascular cuffing in the brainstem that were comprised mainly of lymphocytes, monocytes, and plasma cells. Immunohistochemical staining identified the presence of S. neurona merozoites associated with the lesions. This zebra probably died from severe endoparasitism that resulted in malabsorption, weakness, and recumbency rather than from encephalitis associated with S. neurona merozoites. Equine protozoal myeloencephalitis has not been reported previously in nondomestic equids.

L25 ANSWER 15 OF 23 MEDLINE

DUPLICATE 8

- AN 2000152631 MEDLINE
- DN 20152631 PubMed ID: 10690772
- TI Improvement of western blot test specificity for detecting equine serum antibodies to Sarcocystis neurona
- AU Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C 2nd; Fox J C
- CS Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.
- SO JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (2000 Jan) 12 (1) 28-32. Journal code: 9011490. ISSN: 1040-6387.
- CY United States

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Journal; Article; (JOURNAL ARTICLE)
DT
LΑ
     English
FS
     Priority Journals
     200003
EΜ
     Entered STN: 20000330
ED
     Last Updated on STN: 20000330
     Entered Medline: 20000321
     Equine protozoal myeloencephalitis (EPM) is a neurological disease of
AB
    horses and ponies caused by the apicomplexan protozoan parasite
     Sarcocystis neurona. The purposes of this study were to
    develop the most stringent criteria possible for a positive test result,
     to estimate the sensitivity and specificity of the EPM Western blot
     antibody test, and to assess the ability of bovine
     antibodies to Sarcocystis cruzi to act as a blocking agent to
    minimize false-positive results in the western blot test for S. neurona.
     Sarcocystis neurona merozoites harvested from equine
     dermal cell culture were heat denatured, and the proteins were separated
    by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20%
    linear gradient gel. Separated proteins were electrophoretically
     transferred to polyvinylidene fluoride membranes and blocked in 1% bovine
     serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6
    horses with S. neurona infections (confirmed by culture from neural
     tissue) and 57 horses without infections (horses from the Eastern
    Hemisphere, where S. neurona does not exist) were tested by Western blot.
    Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-,
     10.5-, and 10-kD bands. Testing was repeated with another step. Blots
    were treated with bovine S. cruzi antibodies prior to
     loading the equine samples. After this modification of the Western blot
     test, positive infection status was significantly associated with
     reactivity to the 30- and 16-kD bands (P<0.001, Fisher's exact test). The
     S. cruzi antibody-blocked Western blot had a sample sensitivity
    of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking proteins not
     specific to S. neurona and using reactivity to the 30- and 16-kD bands as
     the criterion for a positive test.
L25 ANSWER 16 OF 23 WPIDS (C) 2003 THOMSON DERWENT
AN
     1999-571872 [48] WPIDS
DNN N1999-421433
                        DNC C1999-166894
     Biologically pure culture of equine Neospora, used as source of vaccines
TI
     and diagnostic reagents.
    B04 C06 C07 D16 S03
DC
IN
    BARR, B C; CONRAD, P A; MARSH, A E
     (REGC) UNIV CALIFORNIA
PA
CYC 23
PΤ
                  A1 19990923 (199948)* EN
        RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
         W: AU CA JP
    AU 9931874 A 19991011 (200008)
     US 6071737
                  A 20000606 (200033)
                  A1 20010103 (200102)
     EP 1064550
                                        EN
         R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
     JP 2002509702 W 20020402 (200225)
                                              47p
ADT WO 9947927 A1 WO 1999-US5754 19990316; AU 9931874 A AU 1999-31874
     19990316; US 6071737 A US 1998-42600 19980316; EP 1064550 A1 EP
     1999-913906 19990316, WO 1999-US5754 19990316; JP 2002509702 W WO
     1999-US5754 19990316, JP 2000-537071 19990316
    AU 9931874 A Based on WO 9947927; EP 1064550 A1 Based on WO 9947927; JP
     2002509702 W Based on WO 9947927
PRAI US 1998-42600
                   19980316
        9947927 A UPAB: 19991122
     NOVELTY - Biologically pure culture of equine Neospora, is new.
          DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
```

following:

- (a) detecting antibodies (Ab) specifically reactive with equine Neospora antigens (Ag) by forming an Ab-Ag complex;
- (b) detecting Neospora by forming a complex with an **antibody** (Ab1) specifically reactive with Neospora antigen;
- (c) detecting Neospora-specific nucleic acid (I) by hybridization with a specific oligonucleotide probe; and
- (d) pharmaceutical composition containing equine Neospora immunogen and a carrier.

ACTIVITY - Antiprotozoal.

MECHANISM OF ACTION - Induction of a specific immune response.

USE - Immunogens (optionally expressed from gene therapy vectors) from equine Neospora are used in vaccines for treatment or prevention of Neospora infection in horses and other animals. Neospora is a causative agent of equine protozoal myeloencephalitis (EPM). Detection of Neospora-specific antigens, antibodies or nucleic acid (by usual immunoassay or hybridization tests) is used to diagnose infection. Antibodies (Ab) specific for equine Neospora are used for diagnosis; to select candidate immunogens for vaccine development; to isolate proteins; to screen DNA libraries and as therapeutic /prophylactic agents.

ADVANTAGE - Reagents specific for equine Neospora allow differentiation between equine protozoal myeloencephalitis caused by Neospora and Sarcocystis neurona. These pathogens require different treatments and treatment of Neospora is only effective if applied before the parasite has formed cysts. The vaccines also prevent shedding of oocysts by animals known to be infected. Dwg.0/2

L25 ANSWER 17 OF 23 MEDLINE

AN 2000080043 MEDLINE

DN 20080043 PubMed ID: 10613219

TI Encephalomyelitis associated with a Sarcocystis neurona -like organism in a sea otter.

CM Comment in: J Am Vet Med Assoc. 2000 Feb 1;216(3):329

AU Rosonke B J; Brown S R; Tornquist S J; Snyder S P; Garner M M; Blythe L L

CS Animal Medical Care of Newport, OR 97365, USA.

SO JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION, (1999 Dec 15) 215 (12) 1839-42, 1807.

Journal code: 7503067. ISSN: 0003-1488.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200002

ED Entered STN: 20000229

Last Updated on STN: 20000525

Entered Medline: 20000215

AΒ An adult female sea otter housed for 5 years in an outdoor habitat in an aquarium developed signs of neurologic disease. Bilateral caudal paresis was evident initially and other neurologic signs consistent with CNS disease developed rapidly. Diagnostic work-up included CBC, serum biochemical analyses, determination of serum antibody titers, radiography of the vertebral column, CSF analysis, muscle biopsy, computed tomography of the brain, and assays for mercury, lead, and thiamine. A tentative diagnosis of encephalitis caused by a Sarcocystis neurona-like organism was made on the basis of detection of CSF antibodies by use of Western blot analysis. Response to treatment was not satisfactory and the sea otter was euthanatized. Immunohistochemical staining revealed S neurona-like organisms within foci of inflammation in the brain and spinal cord. This report provides evidence that, for sea otters, there may be a mode of transmission of an S neurona-like organism that does not involve opossums.

Equine protozoare Myeloenzephalitis bei einem importierten Paint-Horse

- AU Weigand, K.; Grabner, A.
- CS Chirurgische Tierklinik der LMU Munchen, Veterinarstr. 13, 80539 Munchen, Germany.
- SO Pferdeheilkunde, (1997) Vol. 13, No. 3, pp. 231-234. 19 ref. ISSN: 0177-7726
- DT Journal
- LA German
- SL English
- AB EPM is reported in a 7-year-old American Paint mare, 6 months after it was imported from Florida, USA, to Germany. The mare was referred to the veterinary clinic at Munich University in late pregnancy with severe neurological disorders characterized by ataxia, facial paresis, dullness and considerable problems with uptake of food and water. Mononuclear pleocytosis and elevated protein and lactate concentrations were detected in cerebrospinal fluid (CSF). Diagnosis of active EPM was confirmed by positive Western Blot reactivity on CSF and detection of antibodies to Sarcocystis neurona. The condition of the mare improved during 3 weeks of drug therapy by trimethoprim sulfonamide and palliative treatment. After parturition, the mare and the foal were discharged in good condition. Treatment with oral trimethoprim sulfonamide for a minimum of 90
- L25 ANSWER 22 OF 23 MEDLINE
- AN 97260250 MEDLINE
- DN 97260250 PubMed ID: 9106345
- TI Equine protozoal myeloencephalitis.

days was continued to avoid relapse of EPM.

- AU MacKay R J
- CS Department of Large Animal Clinical Sciences, University of Florida, College of Veterinary Medicine, Gainesville, USA.
- SO VETERINARY CLINICS OF NORTH AMERICA. EQUINE PRACTICE, (1997 Apr) 13 (1) 79-96. Ref: 72
 Journal code: 8511904. ISSN: 0749-0739.
- CY United States
- DT Journal; Article; (JOURNAL ARTICLE)
 General Review; (REVIEW)
 (REVIEW, TUTORIAL)
- LA English
- FS Priority Journals
- EM 199706
- ED Entered STN: 19970612 Last Updated on STN: 19970612 Entered Medline: 19970603
- AB Equine protozoal myeloencephalitis (EPM) is a common neurologic disease of horses in the Americas. Horses with EPM most commonly have abnormalities of gait, but they also may present with signs of brain disease. The disease ranges in severity from mild lameness to sudden recumbency, and clinical signs usually are progressive. A causative agent,

 Sarcocystis neurona, has been isolated from affected horses, and serologic surveys suggest that approximately 50% of horses in the United States have been exposed. EPM is considered a treatable disease, although the response to antimicrobial treatment often is incomplete. This article highlights new information about the life cycle of S. neurona and reviews the literature regarding diagnosis, clinical signs, and treatment of the disease.
- L25 ANSWER 23 OF 23 MEDLINE

DUPLICATE 11

- AN 93385225 MEDLINE
- DN 93385225 PubMed ID: 8373858
- TI Immunohistochemical study to demonstrate Sarcocystis neurona in equine protozoal myeloencephalitis.

- AU Hamir A N; Moser G; Galligan D T; Davis S W; Granstrom D E; Dubey J P
- CS University of Pennsylvania School of Veterinary Medicine, New Bolton Center, Kennett Square 19348.
- SO JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (1993 Jul) 5 (3) 418-22. Journal code: 9011490. ISSN: 1040-6387.
- CY United States
- DT Journal; Article; (JOURNAL ARTICLE)
- LA English
- FS Priority Journals
- EM 199310
- ED Entered STN: 19931105 Last Updated on STN: 19931105 Entered Medline: 19931021
- A 5-year (1985-1989) retrospective immunohistochemical study was conducted AΒ using an avidin-biotin complex (ABC) immunoperoxidase method to demonstrate Sarcocystis neurona in histologically suspect cases of equine protozoal myeloencephalitis (EPM). Primary antibodies against S. neurona and S. cruzi were utilized for the ABC technique. The findings were compared with those from cases in which the organisms were detected by examination of hematoxylin and eosin (HE)-stained neuronal sections. HE-stained sections detected the presence of the organisms in 20% of the suspect cases; whereas the ABC technique confirmed the presence of S. neurona in 51% and 67% of the cases by S. neurona and S. cruzi antibodies, respectively. A review of clinical case histories showed that 21/47 (45%) of the EPM horses with parasites in the tissue sections had prior treatment with antiprotozoal drugs and/or steroids. Using the test results of S. neurona and S. cruzi as a standard reference, HE test sensitivity based on examination of up to 30 neuronal sections per case was only 25%, and test specificity was 91%.

=> s 19 and sarcocystis neurona
L10 39 L9 AND SARCOCYSTIS NEURONA

=> d bib ab

L11 ANSWER 1 OF 1 MEDLINE

AN 2000152631 MEDLINE

DN 20152631 PubMed ID: 10690772

TI Improvement of western blot test specificity for detecting equine serum antibodies to **Sarcocystis neurona**.

AU Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C 2nd; Fox J C

CS Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.

SO JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (2000 Jan) 12 (1) 28-32. Journal code: 9011490. ISSN: 1040-6387.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200003

ED Entered STN: 20000330 Last Updated on STN: 20000330 Entered Medline: 20000321

Equine protozoal myeloencephalitis (EPM) is a neurological disease of AB horses and ponies caused by the apicomplexan protozoan parasite Sarcocystis neurona. The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to Sarcocystis cruzi to act as a blocking agent to minimize false-positive results in the western blot test for S. neurona. Sarcocystis neurona merozoites harvested from equine dermal cell culture were heat denatured, and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated proteins were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 horses with S. neurona infections (confirmed by culture from neural tissue) and 57 horses without infections (horses from the Eastern Hemisphere, where S. neurona does not exist) were tested by Western blot. Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the equine samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16kD bands (P<0.001, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking proteins not specific to S. neurona and using reactivity to the 30- and 16-kD bands as the criterion for a positive test.

=> d his

(FILE 'HOME' ENTERED AT 14:49:07 ON 15 MAY 2003)

FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS, LIFESCI, CAPLUS' ENTERED AT 14:49:30 ON 15 MAY 2003

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E MANSFIELD LINDA S/AU
             30 S E2-E3
Ll
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L2
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                E ROSSANO M G/AU
             25 S E3
L4
                E MURPHY ALICE J/AU
             15 S E2 OR E3
L_5
                E MURPHY A J/AU
            337 S E3
L6
                E VRABLE RUTH A/AU
             11 S E2 OR E3
1.7
                E VRABLE R A/AU
             23 S E2 OR E3
L8
            496 S L1-L8
L9
             39 S L9 AND SARCOCYSTIS NEURONA
L10
L11
              1 S L10 AND (16 KD OR 30 KD)
=> s 19 and antibod?
L12
            51 L9 AND ANTIBOD?
=> s 110 and antibod?
L13
            26 L10 AND ANTIBOD?
=> dup rem 113
PROCESSING COMPLETED FOR L13
             12 DUP REM L13 (14 DUPLICATES REMOVED)
=> d bib ab 1-12
    ANSWER 1 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
AN
     2003:159207 BIOSIS
DN
     PREV200300159207
     A herd-level analysis of risk factors for antibodies to
TI
     Sarcocystis neurona in Michigan equids.
     Rossano, M. G.; Kaneene, J. B. (1); Marteniuk, J. V.; Banks, B.
ΑU
     D.; Schott, H. C., II; Mansfield, L. S.
     (1) College of Veterinary Medicine, Population Medicine Center, A-109
CS
     Veterinary Medical Center, Michigan State University, East Lansing, MI,
     48824-1314, USA: kaneene@cvm.msu.edu USA
SO
     Preventive Veterinary Medicine, (15 February 2003) Vol. 57, No. 1-2, pp.
     7-13. print.
     ISSN: 0167-5877.
     Article
DT
LΑ
     English
AB
     Equine protozoal myeloencephalitis (EPM) is a neurological disease of
     horses and ponies caused by infection of the central nervous system with
     the protozoan parasite Sarcocystis neurona. A
     herd-level analysis of a cross-sectional study of serum antibodies
     to S. neurona in Michigan equids was conducted, using data collected in
     1997 for study that included 1121 equids from 98 Michigan horse farms. Our
     objective was to identify specific herd-level risk factors associated with
     seropositivity. We tested associations between herd seroprevalence and
     various farm-management practices (including feed-storage methods and
     wildlife control). Multivariable models were developed for three strata
     based on relative opossum abundance (opossum districts). Herd
     seroprevalence ranged from 0 to 100% (median = 57%); No risk factor was
     significantly associated with herd seroprevalence at P ltoreg 0.05 in all
     opossum districts. Our results suggest that equids living in areas with
     large opossum populations might be infected with S. neurona from multiple
```

sources.

- L14 ANSWER 2 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
- AN 2003:67219 BIOSIS
- DN PREV200300067219
- TI Immunoassay for equine protozoal myeloencephalitis in horses.
- AU Mansfield, Linda S.; Murphy, Alice J. (1); Rossano, Mary G.
- CS (1) St. Johns, MI, USA USA
 ASSIGNEE: Board of Trustees of Michigan State University
- PI US 6489148 December 03, 2002
- Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 3 2002) Vol. 1265, No. 1, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
- DT Patent
- LA English
- AB An immunoassay for Sarcocystis neurons antibodies in equines is described. The immunoassay uses blocking of Sarcocystis antigens by antibodies to Sarcocystis sp. other than Sarcocystis neurona in connection with the immunoassay.
- L14 ANSWER 3 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
- AN 2002:234586 BIOSIS
- DN PREV200200234586
- TI Antigen test to detect equine protozoal myeloencephalitis in horse serum and cerebrospinal fluid.
- AU Mansfield, Linda S.; Rossano, Mary G.; Murphy, Alice J.; Vrable, Ruth A. (1)
- CS (1) Williamston, MI USA
 ASSIGNEE: Board of Trustees of Michigan State University, East Lansing,
 MI, USA
- PI US 6344337 February 05, 2002
- Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 5, 2002) Vol. 1255, No. 1, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
- DT . Patent
- LA English
- The present invention provides an immunoassay to detect identifying antigens in horses that are infected with Sarcocystis neurona. The immunoassay is preferably an antigen-capture-based assay that relies upon polyclonal or monoclonal antibodies against a 16 (+-4) and/or 30 (+-4) kDa antigens specific to Sarcocystis neurona to detect the presence of the 16 (+-4) and/or 30 (+-4) kDa antigens in equine serum or equine cerebrospinal fluid.
- L14 ANSWER 4 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
- AN 2002:556058 BIOSIS
- DN PREV200200556058
- TI Cross-sectional study of serum antibodies against Sarcocystis neurona in cats tested for antibodies against Toxoplasma gondii.
- AU Rossano, Mary G.; Murphy, Alice J.; Vrable, Ruth
 A.; Vanzo, Nicole E.; Lewis, Stacy K.; Sheline, Katherine D.;
 Kaneene, John B.; Mansfield, Linda S. (1)
- CS (1) Animal Health Diagnostic Laboratory, College of Veterinary Medicine, Michigan State University, East Lansing, MI, 48824 USA
- Journal of the American Veterinary Medical Association, (August 15, 2002) Vol. 221, No. 4, pp. 511-514. http://www.avma.org.print.ISSN: 0003-1488.

MECHANISM OF ACTION - Vaccine.

USE - The vaccines and methods are used for protecting equids against infections by the protozoan parasite Sarcocystis neurona. $\mbox{Dwg.0/0}$

L14 ANSWER 6 OF 12 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

AN 2001-07735 BIOTECHDS

TI Vaccinating equids against protozoal Sarcocystis neurona infections using antigens;

eurona infections using antigens,

Sarcocystis neurona nucleic acid vaccine and recombinant vaccine

AU Mansfield L S; Rossano M G; Murphy A J;

Vrable R A

PA Univ.Michigan-State

LO East Lansing, MI, USA.

PI WO 2001015708 8 Mar 2001

AI WO 2000-US24221 31 Aug 2000

PRAI US 2000-513086 24 Feb 2000; US 1999-152193 2 Sep 1999

DT Patent

AB

LA English

OS WPI: 2001-218486 [22]

A method for vaccinating equids against Sarcocystis neurona infection is claimed. It involves using protein groups of unique 16(+4) or 30(+4) antigens of S. neurona. Also claimed are: a vaccine (I) for providing passive immunity to Sarcocystis neurona infection; a vaccine (II) for active immunization of an equid against a S. neurona infection; a vaccine (III) for protecting an equid from S. neuronna infection; a method (IV or V) for vaccinating an equid against a S. neurona infection; a method (VI) of providing passive immunity to a S. neurona infection; a method (VII) for producing a protein (e.g. glutathione-transferase); a method (VIII) for producing an antibody; providing a microorganism in a culture containing DNA encoding a fusion protein; a monoclonal antibody (IX); an isolated DNA (X); a bacterial clone (XI); a vaccine (XII) for an equid containing an isolated recombinant protein; a vaccine (XIII or XIV) for an equid containing recombinant virus vector containing DNA; and a method (XV) for protecting an equid against S. neurona. The vaccines and methods are used for protecting equids against infection by the protozoon parasite Srcocystis neurona. (54pp)

- L14 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
- AN 2001:135932 BIOSIS
- DN PREV200100135932
- TI The seroprevalence of antibodies to Sarcocystis neurona in Michigan equids.
- AU Rossano, M. G.; Kaneene, J. B. (1); Marteniuk, J. V.; Banks, B. D.; Schott, H. C., II; Mansfield, L. S.
- CS (1) Population Medicine Center, College of Veterinary Medicine, A-109 Veterinary Medical Center, Michigan State University, East Lansing, MI, 48824-1314: kaneene@cvm.msu.edu USA
- SO Preventive Veterinary Medicine, (29 January, 2001) Vol. 48, No. 2, pp. 113-128. print. ISSN: 0167-5877.
- DT Article
- LA English
- SL English
- AB A cross-sectional study of serum antibodies to
 Sarcocystis neurona (the etiologic agent of equine
 protozoal myeloencephalitis, EPM) was performed on Michigan equids. Our
 objectives were to determine the seroprevalence of antibodies to
 S. neurona in Michigan equids and to identify specific risk factors for
 seropositivity. A random, weighted sample of Michigan horse farms

conjugate and then detected by reaction with an appropriate color forming substrate. The **antibody** is immobilized on a support chosen from a membrane or a plate. (64pp)

L14 ANSWER 12 OF 12 MEDLINE

DUPLICATE 7

AN 2000152631 MEDLINE

DN 20152631 PubMed ID: 10690772

TI Improvement of western blot test specificity for detecting equine serum antibodies to Sarcocystis neurona.

AU Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C 2nd; Fox J C

CS Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.

SO JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (2000 Jan) 12 (1) 28-32. Journal code: 9011490. ISSN: 1040-6387.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200003

ED Entered STN: 20000330 Last Updated on STN: 20000330 Entered Medline: 20000321

Equine protozoal myeloencephalitis (EPM) is a neurological disease of AB horses and ponies caused by the apicomplexan protozoan parasite Sarcocystis neurona. The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to Sarcocystis cruzi to act as a blocking agent to minimize false-positive results in the western blot test for S. neurona. Sarcocystis neurona merozoites harvested from equine dermal cell culture were heat denatured, and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated proteins were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 horses with S. neurona infections (confirmed by culture from neural tissue) and 57 horses without infections (horses from the Eastern Hemisphere, where S. neurona does not exist) were tested by Western blot. Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the equine samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-kD bands (P<0.001, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking proteins not specific to S. neurona and using reactivity to the 30- and 16-kD bands as the criterion for a positive test.

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(FILE 'HOME' ENTERED AT 14:49:07 ON 15 MAY 2003)

FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS, LIFESCI, CAPLUS' ENTERED AT 14:49:30 ON 15 MAY 2003

E MANSFIELD LINDA S/AU

L1 30 S E2-E3

E MANSFIELD L S/AU

L2 139 S E3

Rossano, Mary G.

(1) St. Johns, MI, USA USA CS ASSIGNEE: Board of Trustees of Michigan State University

US 6489148 December 03, 2002 PΙ

Official Gazette of the United States Patent and Trademark Office Patents, SO (Dec. 3 2002) Vol. 1265, No. 1, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.

Patent DT

English LA

- An immunoassay for Sarcocystis neurons antibodies in equines is described. AB The immunoassay uses blocking of Sarcocystis antigens by antibodies to Sarcocystis sp. other than Sarcocystis neurona in connection with the immunoassay.
- ANSWER 3 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. L15

2002:234586 BIOSIS AN

PREV200200234586 DN

- Antigen test to detect equine protozoal myeloencephalitis in horse serum TI and cerebrospinal fluid.
- Mansfield, Linda S.; Rossano, Mary G.; Murphy, ΑU Alice J.; Vrable, Ruth A. (1)
- (1) Williamston, MI USA CS ASSIGNEE: Board of Trustees of Michigan State University, East Lansing, MI, USA

US 6344337 February 05, 2002 PΤ

Official Gazette of the United States Patent and Trademark Office Patents, SO (Feb. 5, 2002) Vol. 1255, No. 1, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html.e-file. ISSN: 0098-1133.

Patent DT

English LΑ

- The present invention provides an immunoassay to detect identifying AΒ antigens in horses that are infected with Sarcocystis neurona. The immunoassay is preferably an antigen-capture-based assay that relies upon polyclonal or monoclonal antibodies against a 16 (+-4) and/or 30 (+-4) kDa antigens specific to Sarcocystis neurona to detect the presence of the 16 (+-4) and/or 30 (+-4) kDa antigens in equine serum or equine cerebrospinal fluid.
- L15 ANSWER 4 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE

2002:556058 BIOSIS AN

PREV200200556058 DN

- Cross-sectional study of serum antibodies against Sarcocystis neurona in cats tested for antibodies against Toxoplasma gondii.
- Rossano, Mary G.; Murphy, Alice J.; Vrable, Ruth A.; Vanzo, Nicole E.; Lewis, Stacy K.; Sheline, Katherine D.; Kaneene, John B.; Mansfield, Linda S. (1)
- (1) Animal Health Diagnostic Laboratory, College of Veterinary Medicine, CS Michigan State University, East Lansing, MI, 48824 USA
- Journal of the American Veterinary Medical Association, (August 15, 2002) SO Vol. 221, No. 4, pp. 511-514. http://www.avma.org. print. ISSN: 0003-1488.

DT Article

English LΑ

Objective-To determine apparent seroprevalence of antibodies against AB Sarcocystis neurona in a population of domestic cats previously tested for antibodies against Toxoplasma gondii. Design-Cross-sectional study. Sample Population-Serum from 196 domestic cats. Procedure-Banked serum samples submitted to the Michigan State University Animal Health Diagnostic Laboratory for T gondii diagnostic testing were tested for antibodies against S neurona by use of an indirect reacted with peroxidase conjugate and then detected by reaction with an appropriate color forming substrate. The antibody is immobilized on a support chosen from a membrane or a plate. (64pp)

L15 ANSWER 14 OF 15 MEDLINE

DUPLICATE 8

- AN 2000152631 MEDLINE
- DN 20152631 PubMed ID: 10690772
- TI Improvement of western blot test specificity for detecting equine serum antibodies to **Sarcocystis neurona**.
- AU Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C 2nd; Fox J C
- CS Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.
- SO JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (2000 Jan) 12 (1) 28-32. Journal code: 9011490. ISSN: 1040-6387.
- CY United States
- DT Journal; Article; (JOURNAL ARTICLE)
- LA English
- FS Priority Journals
- EM 200003
- ED Entered STN: 20000330 Last Updated on STN: 20000330 Entered Medline: 20000321
- Equine protozoal myeloencephalitis (EPM) is a neurological disease of AB horses and ponies caused by the apicomplexan protozoan parasite Sarcocystis neurona. The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to Sarcocystis cruzi to act as a blocking agent to minimize false-positive results in the western blot test for S. neurona. Sarcocystis neurona merozoites harvested from equine dermal cell culture were heat denatured, and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated proteins were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 horses with S. neurona infections (confirmed by culture from neural tissue) and 57 horses without infections (horses from the Eastern Hemisphere, where S. neurona does not exist) were tested by Western blot. Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the equine samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-kD bands (P<0.001, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking proteins not specific to S. neurona and using reactivity to the 30- and 16-kD bands as the criterion for a positive test.
- L15 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
- AN 2000:21537 BIOSIS
- DN PREV200000021537
- TI Simplified technique for isolation, excystation, and culture of Sarcocystis species from opossums.
- AU Murphy, A. J. (1); Mansfield, L. S.
- CS (1) Animal Health Diagnostic Laboratory, Michigan State University, East Lansing, MI, 48824 USA
- SO Journal of Parasitology, (Oct., 1999) Vol. 85, No. 5, pp. 979-981. ISSN: 0022-3395.
- DT Article

and analyzed both qualitatively (western blot) and quantitatively (anti-17kDa) for anti-S. neurona IgG. Four of the challenged horses were given dexamethasone (0.1mg/kg orally once daily) for the duration of the experiment. All challenged horses immunoconverted against S. neurona in blood within 32 days of challenge and in CSF within 61 days. There was a trend (P = 0.057) for horses given dexamethasone to immunoconvert earlier than horses that were not immunosuppressed. Anti-17kDa was detected in the CSF of all challenged horses by day 61. This response was statistically greater at day 32 in horses given dexamethasone. Control horses remained seronegative throughout the period in which all challenged horses converted. One control horse immunoconverted in blood at day 75 and in CSF at day 89. Signs of neurologic disease were mild to equivocal in challenged horses. Horses given dexamethasone had more severe signs of limb weakness than did horses not given dexamethasone; however, we could not determine whether these signs were due to spinal cord disease or to effects of systemic illness. At necropsy, mild-moderate multifocal qliosis and neurophagia were found histologically in the spinal cords of 7/8 challenged horses. No organisms were seen either in routinely processed sections or by immunohistochemistry. Although neurologic disease comparable to naturally occurring equine protozoal myeloencephalitis (EPM) was not produced, we had clear evidence of an immune response to challenge both systemically and in the CNS. Broad immunosuppression with dexamethasone did not increase the severity of histologic changes in the CNS of challenged horses. Future work must focus on defining the factors that govern progression of inapparent S. neurona infection to EPM.

- L20 ANSWER 3 OF 3 MEDLINE
- MEDLINE AN93222344
- DN 93222344 PubMed ID: 8466988
- ΤI Equine protozoal myeloencephalitis: antigen analysis of cultured Sarcocystis neurona merozoites.
- Granstrom D E; Dubey J P; Davis S W; Fayer R; Fox J C; Poonacha K B; Giles AU R C; Comer P F
- Department of Veterinary Science, University of Kentucky, Lexington CS 40546-0099.
- JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (1993 Jan) 5 (1) 88-90. SO Journal code: 9011490. ISSN: 1040-6387.
- CY United States
- DTJournal; Article; (JOURNAL ARTICLE)
- LΑ English
- FS Priority Journals
- EΜ 199305
- ED Entered STN: 19930521

Last Updated on STN: 19930521

Entered Medline: 19930510 AΒ

Antigens of cultured Sarcocystis neurona merozoites were examined using immunoblot analysis. Blotted proteins were probed with S. cruzi, S. muris, and S. neurona antisera produced in rabbits, S. fayeri (pre- and post-infection) and S. neurona (pre- and post-inoculation) sera produced in horses, immune sera from 7 histologically confirmed cases of equine protozoal myeloencephalitis (EPM), and pre-suckle serum from a newborn foal. Eight proteins, 70, 24, 23.5, 22.5, 13, 11, 10.5, and 10 Kd, were detected only by S. neurona antiserum and/or immune serum from EPM-affected horses. Equine sera were titered by the indirect immunofluorescent antibody (IFA) method using air-dried, cultured S. neurona merozoites. Anti-Sarcocystis IFA titers were found in horses with or without EPM. Serum titers did not correspond to the number of specific bands recognized on immunoblots.

Entered Medline: 20020117

A two-month-old Appaloosa colt developed neurological signs shortly after AB birth involving deficits affecting cranial nerves IV, VII, VIII, IX, X and XII, and possibly nerve VI. The most likely differential diagnoses were congenital anomalies, meningoencephalitides, trauma or nutritional causes. The foal was investigated by the analysis of cerebrospinal fluid (CSF), electromyelography (EMG), brain auditory evoked responses, magnetic resonance imaging (MRI), peripheral nerve biopsy, and Western blot analysis for the presence of intrathecal antibodies to Sarcocystis neurona, the causative agent of equine protozoal myeloencephalitis. Significantly abnormal EMG findings included spontaneous electrical activity of the tongue, suggesting denervation. The MRI was useful in ruling out masses, congenital anomalies and focal abscessation. The cytology of CSF revealed mild mononuclear reactivity. Western blot testing of CSF was positive, indicating the intrathecal presence of antibodies to S neurona. The foal was treated with pyrimethamine and trimethoprim-sulphadiazine for two months and returned to nearly normal neurologic status.

- L25 ANSWER 10 OF 23 CABA COPYRIGHT 2003 CABI
- AN 2001:140175 CABA
- DN 20013139273
- TI Efficacy of ponazuril 15% oral paste as a **treatment** for equine protozoal myeloencephalitis
- AU Furr, M.; Kennedy, T.; MacKay, R.; Reed, S.; Andrews, F.; Bernard, B.; Bain, F.; Byars, D.
- CS Virginia-Maryland Regional College of Veterinary Medicine, Marion duPont Scott Equine Medical Center, PO Box 1938, Leesburg, VA 20177, USA.
- SO Veterinary Therapeutics, (2001) Vol. 2, No. 3, pp. 215-222. 15 ref. ISSN: 1528-3593
- DT Journal
- LA English
- Equine protozoal myeloencephalitis (EPM) is a neurologic disease of horses AΒ most commonly caused by the protozoan parasite Sarcocystis neurona. Until recently the only treatment option was the combination of a sulfonamide with pyrimethamine. The present study was performed to assess the efficacy of ponazuril, an anticoccidial triazine-based compound, as a treatment for naturally occurring EPM. 101 horses with EPM were randomly allocated to treatment with ponazuril 15% oral paste at either 5 or 10 mg/kg body weight for 28 consecutive days. Horses were evaluated clinically and by analysis of blood and cerebrospinal fluid (CSF) before and 28 and 118 days after the start of treatment. Clinical success was defined as either an improvement in neurologic score by at least one grade (on a 0 to 5 scale) or conversion to negative status on Western blot for S. neurona antibodies by 20 days following cessation of treatment. Overall, 62% of the bones, including 28 of 47 treated with ponazuril at 5 mg/kg and 35 of 54 treated with 10 mg/kg, met the criteria for successful treatment. The Western blot for CSF became negative in 10% (10/101) of the horses. Quantification of the anti-17kDa antibody response in Western blot (relative quantity CSF) did not reveal a significant change in response to treatment . However, immunoglobulin index did decrease significantly during treatment (P=.01). The findings of this study support the efficacy of ponazuril for the treatment of EPM.
- L25 ANSWER 11 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
- AN 2001:169888 BIOSIS
- DN PREV200100169888
- TI Utilization of stress in the development of an equine model for equine protozoal myeloencephalitis.
- AU Saville, W. J. A. (1); Stich, R. W.; Reed, S. M.; Njoku, C. J.; Oglesbee,

- M. J.; Wunschmann, A.; Grover, D. L.; Larew-Naugle, A. L.; Stanek, J. F.; Granstrom, D. E.; Dubey, J. P.
- CS (1) Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University, Columbus, OH, 43210: saville.4@osu.edu
- SO Veterinary Parasitology, (26 February, 2001) Vol. 95, No. 2-4, pp. 211-222. print. ISSN: 0304-4017.
- DT Article
- LA English
- SL English
- AB Neurologic disease in horses caused by Sarcocystis neurona is difficult to diagnose, treat, or prevent, due to the lack of knowledge about the pathogenesis of the disease. This in turn is confounded by the lack of a reliable equine model of equine protozoal myeloencephalitis (EPM). Epidemiologic studies have implicated stress as a risk factor for this disease, thus, the role of transport stress was evaluated for incorporation into an equine model for EPM. Sporocysts from feral opossums were bioassayed in interferon-gamma gene knockout (KO) mice to determine minimum number of viable S. neurona sporocysts in the inoculum. A minimum of 80,000 viable S. neurona sporocysts were fed to each of the nine horses. A total of 12 S. neurona antibody negative horses were divided into four groups (1-4). Three horses (group 1) were fed sporocysts on the day of arrival at the study site, three horses were fed sporocysts 14 days after acclimatization (group 2), three horses were given sporocysts and dexamethasone 14 days after acclimatization (group 3) and three horses were controls (group 4). All horses fed sporocysts in the study developed antibodies to S. neurona in serum and cerebrospinal fluid (CSF) and developed clinical signs of neurologic disease. The most severe clinical signs were in horses in group 1 subjected to transport stress. The least severe neurologic signs were in horses treated with dexamethasone (group 3). Clinical signs improved in four horses from two treatment groups by the time of euthanasia (group 1, day 44; group 3, day 47). Post-mortem examinations, and tissues that were collected for light microscopy, immunohistochemistry, tissue cultures, and bioassay in KO mice, revealed $% \left(1\right) =\left(1\right) \left(1\right) \left$ no direct evidence of S. neurona infection. However, there were lesions compatible with S. neurona infection in horses. The results of this investigation suggest that stress can play a role in the pathogenesis of EPM. There is also evidence to suggest that horses in nature may clear the organism routinely, which may explain the relatively high number of normal horses with CSF antibodies to S. neurona compared to the prevalence of EPM.
- L25 ANSWER 12 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
- AN 2001:169887 BIOSIS
- DN PREV200100169887
- TI Immunoconversion against **Sarcocystis neurona** in normal and dexamethasone-**treated** horses challenged with S. neurona sporocysts.
- AU Cutler, Tim J.; MacKay, Robert J. (1); Ginn, Pamela E.; Gillis, Karen; Tanhauser, Susan M.; LeRay, Erin V.; Dame, John B.; Greiner, Ellis C.
- CS (1) Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32610: mackayr@mail.vetmed.ufl.edu USA
- SO Veterinary Parasitology, (26 February, 2001) Vol. 95, No. 2-4, pp. 197-210. print.
 ISSN: 0304-4017.
- DT Article
- LA English
- SL English
- AB Equine protozoal myeloencephalitis is a common neurologic disease of

horses in the Americas usually caused by Sarcocystis neurona. To date, the disease has not been induced in horses using characterized sporocysts from Didelphis virginiana, the definitive host. S. neurona sporocysts from 15 naturally infected opossums were fed to horses seronegative for antibodies against S. neurona. Eight horses were given 5 X 105 sporocysts daily for 7 days. Horses were examined for abnormal clinical signs, and blood and cerebrospinal fluid were harvested at intervals for 90 days after the first day of challenge and analyzed both qualitatively (western blot) and quantitatively (anti-17 kDa) for anti-S. neurona IgG. Four of the challenged horses were given dexamethasone (0.1 mg/kg orally once daily) for the duration of the experiment. All challenged horses immunoconverted against S. neurona in blood within 32 days of challenge and in CSF within 61 days. There was a trend (P = 0.057) for horses given dexamethasone to immunoconvert earlier than horses that were not immunosuppressed. Anti-17 kDa was detected in the CSF of all challenged horses by day 61. This response was statistically greater at day 32 in horses given dexamethasone. Control horses remained seronegative throughout the period in which all challenged horses converted. One control horse immunoconverted in blood at day 75 and in CSF at day 89. Signs of neurologic disease were mild to equivocal in challenged horses. Horses given dexamethasone had more severe signs of limb weakness than did horses not given dexamethasone; however, we could not determine whether these signs were due to spinal cord disease or to effects of systemic illness. At necropsy, mild-moderate multifocal gliosis and neurophagia were found histologically in the spinal cords of 7/8 challenged horses. No organisms were seen either in routinely processed sections or by immunohistochemistry. Although neurologic disease comparable to naturally occurring equine protozoal myeloencephalitis (EPM) was not produced, we had clear evidence of an immune response to challenge both systemically and in the CNS. Broad immunosuppression with dexamethasone did not increase the severity of histologic changes in the CNS of challenged horses. Future work must focus on defining the factors that govern progression of inapparent S. neurona infection to EPM.

- L25 ANSWER 13 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
- AN 2000:509421 BIOSIS
- DN PREV200000509421
- TI Evaluation of risk factors associated with clinical improvement and survival of horses with equine protozoal myeloencephalitis.
- AU Saville, William J. (1); Morley, Paul S. (1); Reed, Stephen M.; Granstrom, David E.; Kohn, Catherine W.; Hinchcliff, Kenneth W.; Wittum, Thomas E. (1)
- CS (1) Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University, Columbus, OH, 43210 USA
- SO Journal of the American Veterinary Medical Association, (October 15, 2000) Vol. 217, No. 8, pp. 1181-1185. print. ISSN: 0003-1488.
- DT Article
- LA English
- SL English
- AB Objective: To investigate risk factors for use in predicting clinical improvement and survival of horses with equine protozoal myeloencephalitis (EPM). Design: Longitudinal epidemiologic study. Animals: 251 horses with EPM. Procedure: Between 1992 and 1995, 251 horses with EPM were admitted to our facility. A diagnosis of EPM was made on the basis of neurologic abnormalities and detection of antibody to Sarcocystis neurona or S neurona DNA in CSF. Data were obtained from hospital records and through telephone follow-up interviews. Factors associated with clinical improvement and survival were analyzed, using multivariable logistic regression. Results: The likelihood of clinical improvement after diagnosis of EPM was lower in horses used for breeding and pleasure activities. Treatment for EPM increased the probability that a

L25 ANSWER 18 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE

- 1999:283751 BIOSIS AN
- DN PREV199900283751
- ΤI Initial experiences with the use of nitazoxanide in the treatment of equine protozoal encephalitis in Northern California.
- Vatistas, Nicholas (1); Fenger, Clara; Palma, Kathleen; Sifferman, Roger AU
- (1) VM Surgical and Radiological Sciences, University of California, CS Tupper Hall, Room 2112, Davis, CA, 95688 USA
- Equine Practice, (May, 1999) Vol. 21, No. 5, pp. 18-21. SO ISSN: 0162-8941.
- DΤ Article
- English LΑ
- SL English
- AB Equine protozoal myeloencephalitis (EPM) is the most common neurological condition affecting horses in North and South America. Nitazoxanide has been reported to be effective against a wide variety of parasites and bacteria in both animals and humans, including protozoa, nematodes, cestodes, trematodes, almost all anaerobic obligate and facultative bacteria, and some aerobic bacteria. The purpose of this study was to determine the effectiveness of nitazoxanide for the treatment of EPM in horses. For inclusion in the study, horses had to have evidence of proprioceptive deficits in one or more limbs, and have a positive immunoblot (Western blot) assay for Sarcocystis neurona antibodies in cerebrospinal fluid. The degree of ataxia was graded from 0 (none) to 5 (severe). Seven horses fit the criteria for inclusion, five horses were grade 2, one horse was a grade 3, and one horse was a grade 4. Nitazoxanide was administered as a feed additive, as tablets, as a powder, or as a paste at 50 or75 mg/kg for approximately 28 days. Two horses became inappetent and depressed during the course of treatment. However, no long-term sequelae were noted. Four horses became pregnant while on the medication, and remained pregnant at the end of the study period. Neurologic signs returned in two horses, and medication was re-introduced. By the end of the trial (85 to 140 days), five horses were neurologically normal, one horse had improved from a grade 4 to a grade 1, and one horse was unchanged. Cerebrospinal fluid samples were obtained from approximately 85 to 140 days after the start of medication. The samples remained positive for Sarcocystis neurona.antibodies by immunoblot (Western blot). However, in six of the seven horses, the relative quantity of antibody had decreased. In its final formulation as a paste, nitazoxanide was well accepted and well tolerated by horses. It improved the neurological status of six of the seven horses. Nitazoxanide has the advantage over presently available medications in that it is cidal (in other species) rather than static in action, it has been administered to pregnant rodents without inducing signs of fetal abnormalities, and is available in a formulation that is more easily administered to horses.
- L25 ANSWER 19 OF 23 MEDLINE
- 1999048790 MEDLINE AN
- DN 99048790 PubMed ID: 9831950
- ΤI Neospora caninum-associated equine protozoal myeloencephalitis.
- ΑU Hamir A N; Tornquist S J; Gerros T C; Topper M J; Dubey J P
- College of Veterinary Medicine, Oregon State University, Corvallis 97331, CS
- SO VETERINARY PARASITOLOGY, (1998 Nov 27) 79 (4) 269-74. Journal code: 7602745. ISSN: 0304-4017.
- CY Netherlands
- DT Journal; Article; (JOURNAL ARTICLE)
- LA English
- FS Priority Journals
- EΜ 199901

Entered STN: 19990202 ED Last Updated on STN: 19990202 Entered Medline: 19990119

Equine protozoal myeloencephalitis (EPM) was clinically diagnosed in a AΒ The cerebrospinal fluid was 20-year-old horse with severe ataxia. positive for Sarcocystis neurona antibodies by western blot. The horse was administered corticosteroids to facilitate in vitro culture of S. neurona from its spinal cord following necropsy. Microscopic lesions of EPM were present in the brain and in the spinal cord, including multifocal inflammatory cellular infiltrates and several large groups of protozoa. Immunohistochemical, and light and electron microscopic examinations revealed that the protozoa were Neospora caninum and not S. neurona. The protozoa divided by endodyogeny, tachyzoites had rhoptries, and organisms reacted specifically to N. caninum antibodies. Veterinarians should be aware of increasing diagnosis of N. caninum as another etiological agent responsible for the lesions of EPM.

- L25 ANSWER 20 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
- 1997:215661 BIOSIS AN
- PREV199799522165
- Epizootic of equine protozoal myeloencephalitis on a farm. TI
- Fenger, Clara K. (1); Granstrom, David E.; Langemeier, John L.; Stamper, AU Shelby
- (1) Equine Intern. Med. Consulting, 3288 Valhalla Dr., Lexington, KY 40515 CS
- Journal of the American Veterinary Medical Association, (1997) Vol. 210, SO No. 7, pp. 923-927. ISSN: 0003-1488.
- DT Article
- English LΑ Objective-To determine the clinical findings, course of treatment AB , and long-term outcome of horses on a farm in central Kentucky during an epizootic of equine protozoal myeloencephalitis (EPM). Design-Cohort study. Animals-21 horses on a farm in central Kentucky, 12 of which developed clinical signs of EPM. Procedure-Horses on the farm were serially examined for signs of neurologic disease and serum and CSF antibodies to Sarcocystis neurona. Horses were considered to have EPM if they had neurologic signs and positive test results for antibodies to S neurona in CSF. Blood values were monitored for evidence of abnormalities resulting from long-term pyrimethamine and trimethoprim-sulfamethoxazole administration. Physical, neurologic, and fetal necropsy examinations were performed as needed. Horses were treated for EPM until they had negative test results for CSF antibodies to S neurona. Results-Of 21 horses on the farm, 12 had EPM over the course of 6 months. The duration of treatment ranged from 45 to 211 days, excluding 1 horse that persistently had CSF antibodies to S neurona. Adverse effects from pyrimethamine and trimethoprim-sulfamethoxazole administration included transient fever, anorexia, and depression (n = 2); acute worsening of ataxia (2); mild anemia (4); and abortions (3). Clinical Implications-EPM may develop as an epizootic. In the horses of this report, subtle clinical signs that were originally considered unimportant ultimately progressed to obvious neurologic signs. Adverse effects associated with EPM treatment included worsening of neurologic signs, anemia, abortion, and leukopenic and febrile episodes.
- L25 ANSWER 21 OF 23 CABA COPYRIGHT 2003 CABI
- 1998:76244 CABA ΑN
- DN 982206910
- Equine protozoal myeloencephalitis (EPM) in an imported American Paint TIhorse

ANSWER 2 OF 2 MEDLINE

AN 96342244 MEDLINE

DN 96342244

PubMed ID: 8720045

TIToxicity and properties of the extract from Sarcocystis cruzi

Saito M; Taguchi K; Shibata Y; Kobayashi T; Shimura K; Itagaki H ΑU

Kumagaya Meat Inspection Center Saitama Prefecture, Japan. CS

SO JOURNAL OF VETERINARY MEDICAL SCIENCE, (1995 Dec) 57 (6) 1049-51. Journal code: A27; 9105360. ISSN: 0916-7250.

CY

DΤ Journal; Article; (JOURNAL ARTICLE)

LA English

Priority Journals FS

EM199610

Entered STN: 19961022 ED

Last Updated on STN: 19961022

Entered Medline: 19961009

AB The extract from Sarcocystis cruzi cysts in bovine muscle was subcutaneously injected to mice, guinea pigs, chickens, and rabbits to detect its toxicity. Only rabbits showed reactions after administration of the extract at a dose of 25 micrograms. The main clinical signs of the rabbits were depression, reduction in body temperature and intermittent diarrhea and the hematological findings observed were elevation in WBC, RBC, PCV, TP, BUN, AST, AUT and creatinine values and reduction in glucose, K+ and pH of blood. The extract, crude toxin, was a water soluble, acid-alkali stable and thermolabile protein and estimated to be a molecular mass of 15-16 kd.